ANNUAL REPORT

RESEARCH ON NAVY-RELATED COMBAT CASUALTY CARE ISSUES, NAVY OPERATIONAL-RELATED INJURIES AND ILLNESSES AND APPROACHES TO ENHANCE NAVY/MARINE CORPS PERSONNEL COMBAT PERFORMANCE

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Prepared by
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GEO-CENTERS, INC.

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RESEARCH ON NAVY-RELATED COMBAT CASUALTY CARE ISSUES, NAVY OPERATIONAL-RELATED INJURIES AND ILLNESSES AND APPROACHES TO ENHANCED NAVY/MARINE CORPS PERSONNEL COMBAT PERFORMANCE

I. INTRODUCTION

This report summarizes the results of GEO-CENTERS' technical activities for the first option year one of the Naval Medical Research Institute (NMRI) Contract N00014-95-D-0048, Delivery Orders 002 and 003. The delivery orders encompass a variety of scientific studies that are capable of supporting ongoing and projected programs under the cognizance of NMRI; NMRI TOX/DET-Dayton, OH; NMRI/DET-San Antonio, TX; NDRI-Great Lakes, IL; the NDRI Detachment-Bethesda, MD; the National Naval Medical Center-Bethesda, MD; and the U.S. Navy Clothing and Textile Facility-Natick, MA.

The format for these periodic technical progress reports consists of four sections each listed by the location of the research. The sections are (1) Descriptions of work to be performed, (2) Objectives planned for the current reporting period, (3) Summary of work performed during current reporting period, and (4) Objectives for the next reporting period. Accumulated scientific reports, technical reports and journal articles are being provided as part of this quarterly technical progress report. Specifically, the research conducted by GEO-CENTERS during this quarterly reporting period has been focused on the following general scientific programs:

A. Infectious disease threat assessment and preventive medicine programs.
B. Immune cell biology, wound repair and artificial blood studies.
C. Biomedical diving programs.
D. Personnel performance enhancement programs.
E. Breast Care Center.
F. Breast Cancer Research & Education Initiative (BRIN)
G. Directed Energy Effects Research
H. Dental related diseases.
I. Toxicological studies.
J. Human Performance and U.S. Navy Clothing Development

GEO-CENTERS, INC.

II. NMRI, Bethesda, MD

A. INFECTIOUS DISEASE THREAT ASSESSMENT AND PREVENTIVE MEDICINE PROGRAMS

CHRISTIAN, WOHLRABE

Description Of Work To Be Performed

- Provide assistance to the adenovirus surveillance project within the Preventive Medicine Division of Naval Hospital, Great Lakes, IL.
- Assist in the development and implementation of "Operation Stop Cough", a programmatic approach to reducing respiratory illness among Navy recruits.
- Assist with general infectious disease surveillance relevant to recruit respiratory illness.

Technical Objectives For The Reporting Period

- Continue culture surveillance for adenoviral illness among recruits.
- Implement new methods of increasing culture returns from our outpatient clinics.
- Assist with an evaluation of vaccine status and MMR immunity among new recruits.
- Collect metrics on hygiene/handwashing for Operation Stop Cough.
- Continue to liaison between investigators, providers, laboratory staff, and patients to provide adenovirus surveillance information.

Summary Of Work Performed During Current Reporting Period

- Assisted with analysis and control of an outbreak of adenoviral illness in recruits. Provided rapid response, assessment, and vaccine to protect over 10,000 recruits from illness.
- Continued to distribute, collect, and arrange mail-out of culture materials for adenovirus surveillance. Provided astute quality control on culture media.
- Provided assistance to medical professionals in obtaining adenovirus cultures.
- Reviewed records and provided quality control for case reports of respiratory illness.
- Inspected handwashing facilities for recruits, as a quality metric for Operation Stop Cough.

- collected and analyzed recruit survey data on handwashing and past MMR vaccination.

Goals/Objectives For Next Reporting Period

- Continue work on the adenovirus surveillance project, modifying procedures as the protocol and recruit needs change.
- Continue to provide data on handwashing for Operation Stop Cough.
- Assist with hygiene control related to streptococcal pharyngitis challenges among recruits.
- Re-evaluate development of video aids to encourage hygiene/handwashing among recruits.
- Assist with development of presentation for the Navy Environmental Health Center on our recent outbreak of adenoviral illness.
- Enter and analyze data on past vaccination/MMR immunity among new recruits.
- Assist with development of a presentation on MMR immunity among Navy recruits.

GERHARD

Description Of Work To Be Performed

- Provide assistance to the Breast Cancer Prevention Initiative (BRIN) program supported by the Department of Defense and run within the Preventive Medicine Division of the Naval Hospital, Great Lakes, IL.

Technical Objectives For The Reporting Period

- Research most recent breast cancer related events -- new drugs, imaging techniques, and educational tools.
- Teach members of the Navy community about breast cancer and breast self-exam.
- Create databases that organize BRIN programs and purchases.
- Assist in development of the Satellite Mammography Program (SMP).
- Assist in planning of the Women's Health Fair.

Summary Of Work Performed During Current Reporting Period

- Completed orientation to Naval Hospital and branch clinics.
- Completed safety training and annual training requirements for health care workers.
- Conducted daily research, via Internet and Medical Library, on most recent breast cancer news.
- Reviewed and rated breast cancer video library for educational effectiveness.
- Made educational packages to distribute at health fairs and information sessions.
- Coordinated breast cancer educational outreach sessions and booths at the Spouse's Day Out, Family Activities Center, and Tri-Plex Gym.
- Attended in creation of SMP patient database.
- Registered SMP patients in the mammography tracking database.
- Created vouchers and educational posters for the SMP.
- Created an inventory tracking system for all BRIN purchases.

Goals/Objectives For Next Reporting Period

- Continue breast cancer research on the Internet and in the Medical Library.
- Continue educational outreach programs.
- Continue marketing BRIN's SMP
- Contact Great Lakes Bulletin (Base Newspaper) about possibly writing educational articles and promoting BRIN programs.
- Start Mail Education Reminder Program and Birthday Card Reminder Program.
- Start breast cancer awareness testing of female recruits.
- Assist with the establishment of the Health Promotion Resource Center

JENDREK

Description Of Work To Be Performed

- Scott Jendrek conducts fermentations in a BL-3 suite and depending on the organism of the fermentation may also perform some or all of the downstream processing associated with the project. He also creates all associated paperwork (standard operating procedures, batch

records, etc.) with the fermenter and related equipment. Scott also does much of the HPLC work towards optimizing current protein purification methods and procedures, as well as some of the Molecular Biology associated with his position.

Technical Objectives For The Reporting Period

- Continue the work with the LF producing strain of *B. anthraces* and then scale that up to the 20 liter level, then Scott will create a purification for LF. When the new HPLC comes in he will perform more of the isoform separations using the Resource Q resin.

Summary Of Work Performed During Current Reporting Period

- The Lethal Factor producing strain of *B. anthraces* was grown and mostly optimized for the 20 liter level, the 'mostly' status is because another instrument is required to measure the available glucose in the vessel. This instrument should be in within the week. Seven straight weeks of fermentations have most likely hammered out the details of what this particular strain needs to produce respectable amounts of the LF protein. Scott will not be developing the purification scheme for LF as first thought. The new HPLC has been installed and is operational. As soon as the LF fermentation is completely optimized Scott will continue his isoform work on Protective Antigen using the resource Q and some new resins that were recently developed that should actually give better results.

Goals/Objectives For Next Reporting Period

- Continue work on the LF production and write a batch record for the fermentation. Will go back to the isoform separation work which was started just prior to the LF project being moved to the front burner.

KERBY

Description of Work to Be Performed

- Senior Scientist; Diagnostic Systems Division, Systems Development Department: develop diagnostic systems to detect and differentiate various viruses and bacteria.

Technical Objectives for the Reporting Period

- Continue with the automated sequence analysis of various viral and bacterial PCR products or clones.
- Test various primers for their usefulness as PCR or sequencing primers.
- Design and synthesize primers and probes for other departments in DSD.
- Continue the cloning of selected genes from these various agents.

Summary Of Work Performed During Current Reporting Period

- Synthesized sequencing and PCR primers for Anthrax, Plague, Q-fever, Poxviruses, Ebola, Hantaan, Dengue, and Shigella.
- Completed the cloning of Variola genes as well as a selection of Vaccinia, Camelpox, Monkeypox, and Cowpox genes.
- Verified the sequences of PCR products or clones of Poxviruses, Y.pestis, B.anthracis, F.tularensis, C.burnetti, S.aureus and other agents.

Publications:
- Manuscript in preparation

Goals/Objectives For Next Reporting Period

- Continue in primer and probe synthesis, automated sequencing analysis, cloning genes, and development of rapid diagnostic systems.

MIHALIC

Description Of Work To Be Performed

- Serves as a laboratory technician, working in the Diagnostic Systems Division at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) at Ft. Detrick, Md. The objective of the division is to develop and optimize assays for the detection of

infectious diseases. My personal responsibilities at the time are in developing and optimizing electrophoretic assays applicable to the overall interests of the division.

Technical Objectives For The Reporting Period

- The past quarter, included a few very important objectives. The first and most important was to conclude the research for a poster and turn in the written material and data to the visual arts department to be mounted on poster board. The next very important goal was to aid in the recovery and purification of a recombinant hantaan nucleocapsid protein. It has been a difficult project and further research was needed to determine the best way to recover the protein. Since the hantaan western blot project was finishing, work began on the Dengue project. With the aim to have an IgM western blot protocol established for that as well. In completing these projects, the advantages of scanner software was incorporated to analyze results and make SDS-PAGE and western blots a more useful tool.

Summary of Work Performed During Current Reporting Period

- In the past quarter, the research for a poster presentation was completed, and will be presented at the American Society of Tropical Medicine and Hygiene Meeting in Orlando, Florida in December. A western blot diagnostic assay for hantaan was developed and the quality control aspects completed to assure the reproducibility of the assay. Also, the purification of the recombinant hantaan nucleocapsid protein has been optimized and that information will also be presented in Orlando as a second poster. Through this purification, techniques for immobilized metal affinity chromatography using the HPLC equipment and chromatography in general were developed. The scanner was useful in making figures for the poster presentations.

Posters:

- "Development of a Chemiluminescent Western Blot for Detecting Hantaan Specific Antibodies." Mihalic, KA, Rossi, CA, Moss, DW, Parker, RW, and Henchal EA. Geo-Centers, Inc.; Diagnostic Systems Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD 21702-5011
- "Production and Purification of a Recombinant Hantaan Nucleocapsid Protein and Its Application in Diagnostic Assays." Moss, DW, Courtney, BC, Feldser, DM, Mihalic, KA, Rossi, CA, Kerby, SB, Phillips, SM, and Henchal, EA.; Diagnostic Systems Division, U.S. Army Medical Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, MD 21702-5011
- "Malaria: A Cause For False-Positive Reactions in IgM Capture ELISAs." Rossi CA, Lewis TE, Mihalic KA, Moss DW, Watts DM, Lucas CM, Kester K, and Henchal EA. Diagnostic Systems Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD; U.S. Naval Medical Research Institute Detachment, Lima, Peru; and Department of Immunology, Walter Reed Army Institute of Research, Washington, DC.

Goals/Objectives For Next Reporting Period

- In the next quarter, it is planned to finish the projects currently in progress and use them to initiate new projects. The western blot assay developed for hantaan, will used to test a panel of sera to obtain more data on the assay. Also, the format of the assay will be helpful in creating other similar diagnostic assays. A large quantity of recombinant hantaan nucleocapsid antigen will be produced for use in many assays. One such assay is a fast, lateral flow assay to detect antibodies in sera. We will try and apply this assay to many different diseases such as hantaan and also plague. Along with finishing that recombinant project, work on the expression and purification of a recombinant ebola protein will begin. Finally, a new technician will be trained in the production of the western blot assay to convert it to a kit assay.

HEAVEY

Description Of Work To Be Performed

- Senior Scientist II

Technical Objectives For The Reporting Period

Orthopoxvirus Project:

- Confirm sequences and transfer the cloned monkeypox virus genes into a VEE replicon for expression in vitro, and confirm expression of proteins in vitro.
- Initiate efforts to obtain variola genes though WHO, which may prove as useful targets for detection of orthopoxviruses in aerosols.

Filovirus Project:

- Evaluate post-challenge serum from guinea pigs which were immunized with VEE replicon expressing MBGV proteins via ELISA to determine if sterile immunity was obtained.
- Immunize a new group of guinea pigs with VEE replicons to examine the ability of individual gene products to protect against a heterologous challenge virus. Specifically, MBGV GP, NP, and VP35 will be used to immunize animals three times at 28 day intervals. After completion of immunization schedule, half the animals will be challenged with a homologous virus isolate and half with a heterologous virus isolate.

Summary Of Work Performed During Current Reporting Period

Orthopox Project:

- Several forms of poxvirus (vaccinia) antigen were produced. These include, cell associated vaccinia, vaccinia extracted with Genetron, and vaccinia extracted with Genetron followed by treatment with trypsin. This antigen was used in preliminary studies to determine if any of the production methods of antigen impact negatively on the ability of selected Mabs to recognize vaccinia virus in an ELISA.
- Cloned several genes from monkeypox virus that may be useful as targets for detection of orthopoxviruses in aerosols. Genes cloned from monkeypox (D8L, L1R, A33R, A34R, and H3L) were sequenced and then subcloned in the VEE replicon. The clones were examined for expression of protein and VEE replicon particle packaged.
- Arrangements were made for access to killed variola antigen at the Centers for Disease Control and Prevention in Atlanta, GA. This antigen will be used to confirm that Mabs which cross react with vaccinia and monkeypox in ELISA also react with variola.

Filovirus Project:

- Continued guinea pig challenge experiments to determine the efficacy of VEE replicon-based vaccines for Marburg virus (MBGV). Three lines of experimentation were begun or continued.
- Strain 13 guinea pigs were immunized with VEE replicons expressing either MBGV GP, GPTM, NP or VP35 3 times at 28 day intervals. For each antigen, animals were divided in half and one half challenged with a homologous virus (MBGV Musoke) and the other half challenged with a heterologous virus (MBGV Ravn), in order to evaluate the ability of replicon vaccination to induce a cross protective response. Experiment is ongoing with results expected in 1-2 weeks.
- Three different strains of guinea pigs were immunized with VEE replicons that expressed either MBGV GP, NP, or VP35. Three immunizations have been administered 28 days apart. Serum antibody tiers have been determined, and animals will be challenged in the near future to determine if there is an effect of genetic background (i.e. MHC type) on protection with any of the antigens listed above.
- Strain 13 guinea pigs have been immunized with VEE replicons expressing MBGV GP, NP, or VP35. Three immunizations have been administered 28 days apart. Serum antibody tiers will be determined. Serum and immune cells will be obtained from these immune animals for transfer into naive strain 13 guinea pigs to indicate whether antibody or cell mediated immunity is the predominant effector of protection.

Goals/Objectives For Next Reporting Period

Orthopoxvirus Project:

- More extensive evaluation of Mabs' ability to recognize antigen produced and treated with Genetron and/or trypsin.
- Production of larger amounts of Mabs which appear to be promising with respect to reaction to variola (and other poxviruses) as a deliverable.
- Start immunizations of animals with VEE replicons which express the individual cloned monkeypox and/or vaccinia genes to generate a wider array of reagents (both polyclonal and monoclonal antibodies) useful for detection of orthopoxviruses in the environment.

Filovirus Project:

- Finish the three existing guinea pig experiments.
- Start immunizations of nonhuman primates with VEE replicon expressing MBGV GP and NP genes to examine protective efficacy in a more relevant model.

II. NMRI, Bethesda, MD

B. IMMUNE CELL BIOLOGY, WOUND REPAIR RESEARCH AND ARTIFICIAL BLOOD PROGRAM

BITENSKY

Description Of Work To Be Performed

- Mark Bitensky is the Principal Investigator who provides technical direction for the Geo-Centers/NMRI blood storage contract. The other scientists on the project, including Dr. Yoshida, respond to Dr. Bitensky, who is a senior research professor in the Boston University College of Engineering.

Technical Objectives For The Reporting Period

- During this period the principal focus has been to understand and address the discrepancies between our *in vitro* survival measurements and the expected performance of our red cell *in vivo* human survival studies. Since our *in vivo* data raise expectations that the oxygenated red blood cells should show excellent properties including *in vivo* survivals following 18-21 weeks of refrigerated storage, and since such expectations are strongly demonstrated by the *in vitro* battery of tests, we are understandably disappointed that we have managed only to prolong our storage when referenced by *in vivo* survivals from the conventional 6 to our current best effort of 9-10 weeks. This discrepancy, we believe, reflects the fact that during prolonged refrigerated storage, several problems arise which interfere with the red cells' circulating functions *in vivo*. These problems are associated with a change in red cell ionic composition and water content, changes in cytosolic pH, and changes in the ionic composition, lactic acid composition, and pH of the bathing medium. We are focusing on developing exchange solutions to correct the changes that occur to the storage solution, as well as changes in the storage solution which are anticipated to interfere with the accumulation of problems during storage. These changes include changing the amount of impermeant ion species in the storage bag, as well as introducing additional amounts of sodium in the storage bag, and small quantities of ammonium ion. We have developed a new microfabricated array which is instrumented by a computer on a silicon chip, and

which behaves as a surrogate capillary. In order to evaluate the elusive parameter of red cell deformability as part of our surrogate battery of tests which we hope will eventually obviate the need for *in vivo* survival measurements.

Summary Of Work Performed During Current Reporting Period

- The principal focus during this last quarter has been to compare *in vivo* and *in vitro* diagnostics over periods of 10 weeks of storage and beyond, to develop the filter and array technologies that would permit us to measure red cell deformability, and to compose and evaluate a series of storage solutions in which sodium ion and mannitol vary. We are also looking at regeneration solutions.

Abstracts:

- Abstract published: Yoshida, T., Lee, J., McDonough, W., Friedman, K., and Bitensky, M.W. (1997) Anaerobic Refrigerated-storage of Red Blood Cells for 9 Weeks: *In Vivo* and *In Vitro* Characteristics. *Transfusion* 37, No. 9s, 104s.

Goals/Objectives For Next Reporting Period

- During this coming reporting period, our principal focus is to yet further improve our abilities to evaluate the microscopical and molecular conditions of the cytoskeleton and lipid bilayer following various storage periods. In addition, we are focusing intensely on our abilities to measure red cell deformability and in our abilities to deoxygenate our red cell storage system with greater efficiency, ease and speed. Our group is now selecting optimal conditions in order to obtain FDA approval for the next round of *in vivo* survival measurements.

GORDON

Description Of Work To Be Performed

- Scientist I and representative of GEO-CENTERS for the NMRI contract. Serves as and performs work as a research assistant. Responsible for implementing and carrying out

aspects of the Navy Blood Storage Project being conducted at Boston University. Responds to Tatsuro Yoshida, Ph.D. and Mark Bitensky, M.S., both whom serve as investigators of the project.

Technical Objectives For The Reporting Period

- The primary objective is to develop assays for *Yersinia enterocolitica*, the bacterium that can live and thrive in stored blood bags. An ongoing objective was to monitor the growth of *Yersinia* in blood bags under various environmental conditions throughout the extended storage period. There are other bacterial strains capable of living in stored blood (*staphylococcus* and *Pseudomonas*) and those organisms were to be cultured and manipulated similarly to *Yersinia.*

Summary Of Work Performed During Current Reporting Period

- *Yersinia enterocolitica* has been monitored in stored blood for several months. Growth rate characteristics, bacterial cell numbers in blood bags, and effect of bacterial presence on storage solution components have been investigated. Continued work has been done on various manipulated growth parameters including temperature, required nutrient levels, and oxygen tension. Ongoing monitoring of blood bags containing *Yersinia* has yielded further insight into the longevity and proliferation of this bacterium in stored blood.
- *Staphylococcus* and *Pseudomonas* have both been introduced to the blood bags for monitoring purposes. Similar studies as mentioned above have been conducted on these organisms in stored blood bags.

Goals/Objectives For Next Reporting Period

- N/A due to funding.

MARK

Description Of Work To Be Performed

- Scientist I and representative of GEO-CENTERS for the NMRI contract. Serves as and performs work as a research assistant. Responsible for implementing and carrying out aspects of the Navy Blood Storage Project being conducted at Boston University. Responds to Tatsuro Yoshida, Ph.D. and Mark Bitensky, M.D., both whom serve as investigators of the project.

Technical Objectives For The Reporting Period

- The primary objective for the reporting period has been to determine the phosphorylation levels of the cytoskeleton of the red blood cell (RBC) in the capillaries. This laboratory has found evidence using a micro-array that simulates the capillaries and allows one to evaluate the red blood cell, that the missing link in understanding how to store the RBCs is in the deformation of the cytoskeleton. RBCs that have been stored for 12 weeks are not able to deform and circulate through the capillaries as well as fresh blood. In order to deform through the capillaries, it has been hypothesized that certain proteins in the cytoskeleton such as spectrin and band-3 are phosphorylated. The phosphorylation adds negative charges to amino acids in the protein which repel and allow the protein to deform. This results in the deformation of the red blood cell. Therefore, the ability to store blood may result from the ability to understand the deformation due to phosphorylation of the red blood cell.

Summary Of Work Performed During Current Reporting Period

- During this reporting period, work was begun on a technique that would facilitate analyzing the phosphorylation levels of the proteins in the red blood cell membrane. The red blood cells were lysed in hypotonic buffer, forming ghosts which consist only of the membrane and cytoskeleton. The cytoskeleton was treated with various enzymes which level off polypeptides that are not embedded in the membrane. These are ideally the same peptides that are available for phosphorylation. Finally, these peptides were run on mass spectrometry and it was determined which cytoskeleton protein they originated from. This method will determine which proteins are phosphorylated and if this phosphorylation level changes under certain circumstances.

Goals/Objectives For Next Reporting Period

- N/A due to funding.

TARR

Description Of Work To Be Performed

- Serves as a protein chemist working on the Navy Blood Storage Project being conducted at Boston University. His main duty is to design, coordinate and supervise experiments in chemical modification of the red cell surface and the analysis of the cell membrane and associated proteins. Dr. Tarr reports to Dr. Mark Bitensky at B.U.

Technical Objectives For The Reporting Period

- Study the chemical glaciation of components on the surface of red blood cells as they affect the various in vitro diagnostics available in this laboratory. The use of glycosyltransferases to decorate oligoglycans on the proteins attached to the outer cell surface was to investigated. Both efforts were directed at prolonging the survival of stored and transfused red cells.
- Research was to continue on the use of mass spectrometry, specifically MALDI-TOF-MS, to analyze relatively unfractionated digests of cytoskeleton, with the aim of establishing an analytical system for natural and induced shifts in this critically important red cell structure.

Summary of Worked Performed During Current Reporting Period

- Compatibility of glaciation chemistry with red blood cell survival was established, though fully favorable diagnostics have not yet been achieved. No fundamental flaw in the design of this method for stabilizing and shielding the red cell from immune and complement recognition was discovered. Progress on the use of enzymes to add sialic acid analogues to the surface of cells was minimal, although similar procedures in other domains have been well established in the literature. There are unresolved issues of compatibility and expense, as well as the design of an effective batch processing procedure.

- The cytoskeleton analysis program has been advanced considerably, with construction of the required database, writing of necessary software to process the very complex mass spectrometric data, and experimental results with five different tools of digestion used under a variety of conditions. It seems clear at this point that some fractionation prior to MALDI-TOF-MS analysis will be necessary in order to concentrate the relevant modified peptides and simplify the data for computerized interpretation. Use of LC-ESI source might be a better approach, though work will continue with the available instrumentation, taking advantage of its sensitivity and rapid processing of multiple samples.

Goals/Objectives For Next Reporting Period

- N/A due to funding

THOMAS

Description Of Work To Be Performed

- Mr. Thomas, as Engineer III, serves as the Computer Aided Design Drafter(CADD) Manager, representing GEO-CENTERS, INC., in support of biomedical research and development activities located at the Walter Reed Army Institute of Research(WRAIR)-Health Facility Planning Agency(HFPA) Office. He is responsible for organizing, managing and maintaining a CADD department and establishing a system of files and directories for working drawings. Mr. Thomas is also responsible for implementing procedures for manipulation of drawing files and developing user(working) drawings from existing documentation of new health facility.

Technical Objectives For The Reporting Period

- The primary objectives during this reporting period included developing user drawings of all floors of new health facility;
- Provide assistance to and coordinate with the Health Facility Planning Office(HFPO) and Corp. Of Engineers(COE) to facilitate updates and revisions to design of new facility;
- Provide technical training to COE, HFPO, Transition Action Team(TAT) and other staff members in utilizing CADD program-MicroStation Review;

- Other objectives include verifying hardware/software needs, determining items needed to further enhance the productivity of CADD department.

Summary Of Work Performed During Current Reporting Period

- Established a substantial quantity of user drawings;
- Generated drawings for HFPO and TAT staff, for use in presentations, meetings and tours of new facility;
- Trained various staff members in the use of CAD package;
- Determined needs and validated necessary purchases of plotter and printer equipment, paper and various other CAD and computer supplies;
- Attended MicroStation 95 conference/seminar to gain further knowledge, skills and tools available in MicroStation to assist in increasing productivity of drawing design.

Goals/Objectives For Next Reporting Period

- Continue to provide support to HFPO/HFPA, Army, Navy, COE and TAT staff;
- Continue to generate working drawings which can be used effectively for space utilization;
- Continue to maintain all CADD systems up and functioning at optimum levels;
- Implement updates to existing contract drawings per changes and revisions made by COE;
- Investigate potential for linking a database and CAD package to create renderings for space management;
- Stay abreast of any new CAD features or processes which may assist in the design of the user drawings and arrange for any further training as necessary.

SHIROKOV

Description Of Work To Be Performed

- Yelizaveta Shirokov is a Scientist I and representative for GEO-CENTERS for the NMRI contract. She serves as and performs work as a research assistant. She is responsible for implementing and carrying out in vitro diagnostic tests of red blood cells stored under oxygen-free conditions for he Navy Blood Storage Project being conducted at Boston

University (BU.) Ms. Shirokov responds to Dr. Tatsuro Yoshida and Dr. Mark Bitensky, both of BU, who serve as investigators of the project.

Technical Objectives For The Reporting Period

- The main objective of this quarter was gain experience with the assays used to determine blood viability (i.e., ATP and hemolysis assay, vesicle assay) on blood stored with the new additive solution (OSAF1) in vitro under anaerobic condition and with values of hematocrit 50 and 30. The objective was to get reproducible results from assay to assay.

Summary Of Work Performed During Current Reporting Period

- In vitro experiments on blood with reduced hematocrit levels that were stored anaerobically with the new storage solution OSAF1 was completed. The viability of the cells was determined using ATP and hemolysis, as well as vesicle assays, and potassium and lactate measurements. The objective of getting reproducible results was obtained, and the results were comparable to those obtained in previous experiments.

Goals/Objectives For Next Reporting Period

- To initiate an in vitro storage with new samples under conditions where blood would be stored with reduced hematocrit (30 and 40), anaerobically, and with modified storage solutions OSAF1 and OSAF3.
- Cell metabolism will be measured weekly. A deoxygenation curve of red cells stored under in vitro and in vivo protocols will also be constructed per weekly measurements. The effect of size of the blood storage bag and the effect that the surface to volume ratio has on oxygen removal kinetics and cell preservation in general will be examined.

YOSHIDA

Description Of Work To Be Performed

- Tatsuro Yoshida is a part time Senior Scientist and representative for GEO-CENTERS for the NMRI contract. He serves as a biochemist / red cell physiologist working on the Navy Blood Storage Project being conducted at Boston University. His main duty is to design, coordinate and supervise experiments carried out by Geo-Centers scientists on the blood storage project at Boston University and at the University of New Mexico with Dr. Bitensky. Tatsuro Yoshida reports to Drs. Mark Bitensky at Boston University and Monty Herron at Geo-Centers.

Technical Objectives For The Reporting Period

- The main objectives of this quarter included analyzing the results from *in vivo* 24 hr survival testing at the University of New Mexico, and, based on this data, formulating the protocols for the next *in vivo* tests. In conjunction with the proposed *in vivo* tests, preparation of the Investigational New Drug amendment for submission to the FDA and the Institutional Review Board at the University of New Mexico was begun. Other objectives included continued establishment of procedures to evaluate rheological properties of stored cells; and devising procedures to restore deformability of red cells after prolonged storage and examine the growth of contaminant micro-organisms under prolonged anaerobic storage conditions.

Summary Of Work Performed During Current Reporting Period

- The results of *in vivo* tests on red blood cells stored under anaerobic conditions (carried out at the University of New Mexico) were presented at the annual meeting of the American Association of Blood Banks on 21 October.
- A new research technician was recruited (Yelizaveta Shirokov), and trained extensively to carry out *in vitro* blood storage and diagnostic measurements.
- A series of blood samples were stored under anaerobic conditions in OFAS3, a new modified additive solution, at various hematocrit levels, for evaluation by *in vitro* diagnostics.

- The deformability of stored red cells were measured using Nucleopore filters. The assessment of nano-fabricated arrays (simulated capillary beds) for use as a diagnostic device to test the deformablility status of stored cells was continued.

Publications, Technical Reports, etc.

- Presentation of results of *in vivo* tests on red blood cells stored under anaerobic conditions (carried out at the University of New Mexico) at the annual meeting of the American Association of Blood Banks (10/21/97).
- Abstract published: Yoshida, T., Lee, J., McDonough, W., Friedman, K., and Bitensky, M.W. (1997) Anaerobic Refrigerated-storage of Red Blood Cells for 9 Weeks: *In Vivo* and *In Vitro* Characteristics. *Transfusion* 37, No. 9s, 104s.

Goals/Objectives For Next Reporting Period

- We will continue to design and fabricate a prototype anaerobic blood storage bag with the oxygen sorbent manufacturer, Multisorb Technologies.
- In the past year, we have focused on elevating ATP concentrations, reducing vesicle production and reducing hemolysis of stored cells in order to achieve storage duration of 9-15 weeks (by developing and testing OFAS1 storage solution). We are now launching a multi-pronged storage experiment to achieve the best possible compromise in optimizing these *in vitro* diagnostics, while, at the same time, achieving and preserving an ideal cell deformability so that the stored cells are in an optimized state for return to circulation. Toward this goal, we have formulated OFAS3 additive solution, and we will continue *in vitro* testing of red cells stored in this solution under anaerobic conditions.
- We plan to file an amendment to the existing IND to the FDA and Institutional Review Board (University of New Mexico) in order to carry out in vivo testing of anaerobic blood stored in AS3 additive solution at reduced hematocrit. We believe that this configuration is the route to obtain deployable 9-week blood in the shortest time.

II. NMRI, Bethesda, MD

C. BIOMEDICAL DIVING RESEARCH

OBOWA

Description Of Work To Be Performed

- Provide technical assistance in the Diving Medicine research laboratory investigating exposure to hyperbaric oxygen (HBO) and its effects on the CNS. Prepare brain and spinal cord tissues for histopathology, histochemistry and immunohistochemistry staining procedures. Responsible for small animal care and welfare. Perform surgical procedures on rats. Insure laboratory is maintained and adequately stocked.

Technical Objectives For The Reporting Period

- Development of a rodent model of spinal cord decompression sickness (DCS). This model will be utilized to evaluate pharmacological interventions for prevention and treatment of DCS in US Navy divers.
- Investigate what role vascular intracellular adhesion molecules may play in central nervous system ischemia/reperfusion injury.
- Study immuno-modulation in pathophysiology of DCS.
- Optimize immunohistochemical staining procedures to apply in DCS models.

Summary Of Work Performed During Current Reporting Period

- Prepared animal CNS tissues for all staining procedures.
- Assisted investigators with dive chamber operation.
- Responsibility for maintenance of laboratory facility and supplies.
- Completed the first phase for rodent DCS modeling. Tissue staining is still ongoing and we will transition some of this work to swine. More rodent DCS experiments are in planning stage.

Goals/Objectives For Next Reporting Period

- Planned decompression studies of vascular adhesion molecules has been very successful and more work is planned in this area. The role of endothelial cell adhesion molecules and cytokines in ischemia reperfusion injury will be evaluated utilizing histochemistry and immunohistochemical techniques, also counter receptors to these molecules on white blood cells will be studied using flow cytometry.
- Blood markers of decompression sickness will be evaluated for usefulness in prediction of decompression outcome in rodents and swine.
- Anticipate 50% completion on the function of vascular adhesion molecules in the spinal cord injury which is currently under development.
- Will begin EEG depth electrode recording in the oxygen toxicity work unit and assist with pharmacological modulation of oxygen induced seizures.
- Begin a pilot study to try to quantify infarct size in a rabbit model of cerebral arterial gas embolism (CAGE). If the model is successful, future studies will be initiated towards the treatment of CAGE.

PORTER

Description Of Work To Be Performed

- Support in the selection and testing of a hyperbaric CO2 analyzer for fleet submarine dry deck shelter use.
- Begin work on new tasking to develop and implement a field test plan for divers air bank sampling on 688 class submarines.
- Assist with other laboratory duties as needed.

Technical Objectives For The Reporting Period

- Continue the testing program for the hyperbaric CO2 analyzers approved for fleet use.
- Support new tasking to develop and implement a field test plan for divers air bank sampling on 688 class submarines.

Summary Of Work Performed During Current Reporting Period

- Six prototype hyperbaric analyzers completed a comprehensive testing program. Testing included months of hyperbaric testing in a laboratory hyperbaric chamber. Testing also involved collecting data from field sites aboard fleet dry deck shelters.
- A report based on the findings with the six prototype units was prepared and sent to NAVSEA.
- As a result of the favorable findings, thirteen new hyperbaric CO2 analyzers were ordered for fleet use. These analyzers where received and tested with a similar, but abbreviated, test plan.
- The new CO2 analyzers completed testing and were sent to US Navy Seal Delivery Vehicle Teams for fleet use.
- Ninety new sample bombs, and sampling equipment, were procured and assembled for 688 air bank test program. Actual testing to begin in FY98
- Air bank samples were collected on two 688 submarines due for DDS overhaul.
- Preformed other laboratory as requested.

Publications, Abstracts, etc.

- Co-authored and constructed a poster presentation "PROPOSED REVISED PROCEDURES FOR SCREENING DIVING AIR FOR DRY DECK SHELTER OPERATIONS" presented at the 1997 Undersea Hyperbaric Medical Society meeting in Cancun, Mexico (abstract attached).

Goals/Objectives For Next Reporting Period

- Continue testing program for dry deck shelter hyperbaric CO2 analyzers that will be issued to Seal Delivery Teams for fleet use.
- Continue work on tasking to develop and implement a field test plan for divers air bank sampling on 688 class submarines.

II. NMRI, Bethesda, MD

D. PERSONNEL PERFORMANCE ENHANCEMENT STUDIES

MCCOWIN

Description Of Work To Be Performed

- Provide management support to the Special Operations Forces Medical Technology Development Program at the Naval Medical Research and Development Command. Duties include reviewing and evaluating medical research proposals, reviewing incremental reports and comparing them with the approved research plans, recommending guidance, and drafting periodic and ad hoc management reports, developing presentation materials and managing financial budget. The scope of research includes all topics withing the Special Operations Forces Medical Technology Development Program. This includes investigations relevant to the treatment of disease, trauma, effects of environmental extremes and treatment for medical support of Special Operations Forces Operations. In addition, from time to time, collect, process and report findings on critical issues which are directly related to other urgent military medical research issues within the purview of the Special operations Forces Medical Technology Development Program.

Technical Objectives For The Reporting Period

- Attend Special Operations Medical Association (SOMA) Meeting and USSOCOM BISC Meeting in Dec 97.
- Collect and evaluate 3rd incremental progress reports.
- Collect monthly obligation and expenditure reports from principal investigators.
- Submit monthly obligation and expenditure reports to SOAC.
- Provide input for the reversion of the USSOCOM Project Reference Book.

Summary Of Work Performed During Current Reporting Period

- Work from reporting period objective section was performed during this reporting period.

Goals/Objectives For Next Reporting Period

- N/A due to funding.

II. NMRI, Bethesda, MD

E. BREAST CARE CENTER

GRIMES, JENKINS, WILLIAMS, MCGEE
Patient Service Representative

Description Of Work To Be Performed

- Process and interview patients, incorporate standard patient registration procedures. Maintain uniform policy for check-in/check-out procedures.
- Collect third party insurance forms on each patient.
- Receive patients and incoming telephone calls/inquiries, determine priorities and refer to proper person/department.
- Ensure that all incomplete patient records and third party forms are corrected or returned to proper staff for completion/correction.
- Set up records and filing system for paperwork associated with each patient record. Ensure that all documents processed are in accordance with department standards and that all forms are in designated order in the patient records. Label files for permanent shadow files.
- Orient new support team members and clinical team staff to office routine.
- Call all no-shows, record reason for not keeping appointment in shadow file and initial.
- Print Composite Health Care System (CHCS) daily schedule and end of day reports. Check end of day report for accuracy.
- ADS System: Educate providers, ensure completeness/accuracy of ADS forms, scan forms.
- Inform Technical Assistant of supply levels.

Technical Objectives For The Reporting Period

- Change patient chart system.
- Modify division of duties based on personnel changes and elimination of rotating positions.
- Streamline and organize front-desk procedures.
- Retrieve and ensure completion of third party insurance forms
- Improve routing and response to incoming telephone calls/inquires
- Use standard registration procedures requiring plastic green card for imprinting all forms pertinent to each patient.

- Coordinate policies for scheduling appointments/procedures for patients calling/walk-ins/consults/cards.
- Streamline physician schedule notification process.
- Refine CHCS daily schedule and end of day reporting.

Summary Of Work Performed During Current Reporting Period

- Divided duties among remaining 3 Patient Service Representative Positions. Trained temporaries.
- Continued organization of front-desk procedures
- Assisted in development of standard operating procedures.
- Processed and interviewed patients through CHCS and designated forms, obtained and updated all patient demographic information and ensured completion of forms.
- Obtained and verified pertinent insurance information utilizing available forms. Obtained third party insurance forms from physicians at end of each visit.
- Required identification card from each patient and imprinted all clinic forms pertinent to that patient.
- Received patients and incoming telephone calls/inquiries, determined priorities and referred to the proper source.
- Explained clinic procedures to patients.
- Retrieved/returned Mammogram films daily.
- Obtain authorization for release of mammogram films from patient, for NNMC file tracking.
- Open monthly clinic schedules and make changes as necessary, based on physician schedule changes.
- Ensured completion of incomplete patient records and third party insurance forms.
- Set up records and maintain filing system for paperwork associated with each patient record. Ensured that all documents processed are in accordance with department standards. Filed all forms in designated order in patient record. Labeled files for permanent shadow files.
- Scheduled and coordinated front desk procedures in accordance with department policy. Identified process problems and helped develop suitable solutions.
- Oriented new support team members and clinical team staff to office routine.
- Participated in team planning to assure team members meet team quality standards. Maintain department standards of productivity.
- Notified physicians the day before they are scheduled for clinic; let them know

approximately how many patients they will have.
- Continue working with the Ambulatory Data System (ADS):

Goals/Objectives For Next Reporting Period

- Complete patient chart conversion process.
- Become more proficient in the use of ADS.
- Given the continuing environment of change and the influx of new employees, we will take this opportunity to scrutinize current processes and increase the efficiency of the front desk area.
- Maintain department standards.
- Become more familiar with TriCare.

KIDWELL

Description Of Work To Be Performed

- Manage and maintain the conference room schedules.
- Order supplies for various departments within the center.
- Manage and maintain the procurement process and database.
- Monitor data collected via CHCS and ADS for accuracy.
- Collect and report monthly man-hour reports.
- Manage patient and physician schedule templates in CHCS.

Technical Objective For The Reporting Period

- Become familiar with Microsoft Access procurement database.
- Obtain additional training in CHCS.
- Participate in the coordination of the patient chart filing system conversion.

Summary Of Work Performed During Current Reporting Period

- Maintained conference room schedules in Schedule+.
- Maintained an adequate supply level.
- Attended a CHCS Desktop training session.
- Became familiar with the Microsoft Access procurement database and scheduled a Microsoft Access training course for December.
- Participated in the coordination of the patient chart filing system conversion and played a major role in the conversion completed to date.

Goals/Objectives For Next Reporting Period

- Become proficient in Microsoft Access.
- Complete the patient chart filing system conversion if chart availability allows.
- Create an internet page for the Breast Care Center.
- Update the Patient Service representative SOP.

RICHMAN

Description Of Work To Be Performed

- Perform technical services including mammograms.
- Assisting in biopsies and ultrasounds.

Technical Objectives For The Reporting Period

- Perform various studies within the department thereby increasing knowledge and experience.
- Broaden understanding of the BCC's procedures and personnel. Expand relationship with BCC.
- Take full advantage of any educational opportunities which may arise as time and schedule permits.
- Continued to increase knowledge of mammography and breast diseases using the doctors as teachers.

- Organize and schedule the next BRCA Education Group
- Continue to recruit and register patients for participation in Tam/4-HPR
- Conduct individual and group presentations for BRCA education/counseling
- Continue to act as liaison between BCC and other governmental/research institutions
- Utilizing Care Manager to identify trends of care in the BCC and to document nursing notes
- Keeping the BCC staff abreast of research issues relevant to patient care and staff development
- Attending seminars/conferences for staff and professional development
- Continue to attend Graduate School to further enhance nursing knowledge
- Participation and case study presentation at BCC staff meetings and multidisciplinary meetings
- Update and maintain protocol log books

Technical Objectives For The Reporting Period

- Utilizing Care Manager to identify trends of care in the BCC and to document nursing notes
- Advertisement for patient education program, i.e. flyers and memos
- Evaluation forms for general education group
- Revise Tam/4-HPR pathway
- Create ovarian screening consults for referrals
- Log book for Oncor Med specimen issues
- Develop method for follow-up post results
- Complete patient information booklet to provide information post education session
- Data collection forms and log-books of patients on protocol
- Excel data for BRCA information

Summary Of Work Performed During Current Reporting Period

- Participated in health fair at WRAMC by setting up a booth for genetic testing.
- Quest speaker at WRAMC's Breast Awareness Day.
- Organized patient charts for BRCA and obtained pathology reports
- Created draft for patient information booklet to provide information post education session
- Completed Tri-fold and booklet for BRCA
- Conducted the first general education group for patients, "Genes and breast Cancer"

Summary Of Work Performed During Current Reporting Period

- Performed a variety of mammograms, stereotactic biopsies, needle localizations and ultrasound procedures.
- Interfaced with mammography doctors to increase knowledge in the areas of mammography and breast disease.
- Continued follow-up for screening mammogram program.
- Passed mammography certification registry on 10/16/97.
- Attended customer relations training workshop at NNMC.
- Worked on patient relations skills.

Goals/Objectives For Next Reporting Period

- Attend Lorad Mammotome equipment in service on 1/8/97.
- Attend an educational mammography seminar.
- Broaden my knowledge of breast diseases and mammography.
- Continue to improve interpersonal skills.

HIGGINS

Description Of Work To Be Performed

- Develop an education program for physicians and nurses on genetics and breast cancer
- Collaborate with NSABP research nurse on menstrual cycle protocol
- Continue to register patients on TAM/4-HPR protocol
- Continue to improve the screening process for patient participation in BCC research
- Continue to keep abreast on breast cancer issues using NCI Current Clips
- Continue to further develop personal computer skills
- Continue to attend seminars/conferences on breast cancer issues and professional nursing issues.
- Contact tumor registry regarding patient identification for BRCA
- Continue BCC chart review to identify high risk and strong family history

- Develop one-sheet format for patient satisfaction survey
- Registered additional patients on TAM/4-HPR and BRCA protocol
- BCC chart review to identify high risk and strong family history
- Attended "Insight Program" in the psychiatric department on management of depression in women.
- Participated in Hastings Center education program for practitioners
- Organized and scheduled the BRCA Education Group
- Conducted individualized and group patient information sessions for BRCA
- Kept the BCC staff abreast of research issues relevant to patient care and staff development
- Attended Graduate School to further enhance nursing knowledge
- Updated and maintained protocol log books
- Updated Tam/4-HPR pamphlets

Goals/Objectives For Next Reporting Period

- Continue to improve the screening process for patient participation in BCC research
- Continue to keep abreast on breast cancer issues using NCI Current Clips
- Continue to attend seminars/conferences on breast cancer issues and professional nursing issues
- Contact tumor registry regarding patient identification for BRCA
- Continue BCC chart review to identify high risk and strong family history
- Become more active in the cultural awareness committees
- Participate in the working group to develop breast cancer genetic information for physicians and nurses
- Prepare information for Dewitt regarding BRCA protocol
- Finalize the dates and organize process for the groups in Jan/Feb.
- Review and document on genetic material received from the National Action Plan on Breast Cancer
- Schedule result sessions for patients and f/u for ovarian screening
- Continue graduate studies

LOUIE

Description Of Work To Be Performed

- Serve as mammographer in the department of radiology at National Naval Medical Center (NNMC).
- Serve as consult for referral cases from outside institutions as well as the Breast Care Center (BCC) here at NNMC. Many of these are complex cases which are sent to NNMC for further evaluation or a second opinion.
- Serve as liaison between the medical staff in the BCC and the mammography section of the radiology department.
- Serve as consultant radiologist for weekly surgical tumor board meetings.
- Supervise the radiology resident assigned to the mammography section of the radiology department.
- Serve as consultant to radiology staff regularly rotating through the mammography section.
- Supervise the mammography technologists to insure that the mammograms meet American College of Radiology (ACR) and Food and Drug Administration, Division of Mammmography (FDA) standards for mammography accreditation.
- Investigate, initiate and participate in the planning of other mammography research projects in which NNMC may be a participant.

Technical Objectives For The Reporting Period

- Continue to follow and further develop the protocols established in the mammography section for evaluating patients with breast abnormalities.
- Continue to perform stereotactic needle core breast biopsies.
- Continue to perform and increase the number of ultrasound guided procedures of the breast, as well as ultrasound scans of the breasts for focal abnormalities.
- Continue to supervise and teach the radiology residents rotating through the mammography section.

Summary Of Work Performed During Current Reporting Period

- Continue to serve as one of the two principal mammographers in the department. I am more frequently on the schedule and read more mammographic studies than any other radiologist assigned to the section. There are 1 to 3 half days each week when I am the only mammographer assigned to read films.
- Continued to perform stereotactic needle core biopsies of the breast as well as needle localizations for surgical excisions, on a regular basis.
- Submitted to the NNMC Investigational Review Board a proposal to demonstrate the feasibility of imaging the breast with fluorodeoxyglucose (FDG) using a gamma camera. This procedure has never been published before, and will involve a collaboration of the nuclear medicine and mammography departments of NNMC. The proposal will be defended in January.
- Increased involvement in teaching the radiology residents as they rotate through the mammography section.
- Continue to identify interesting cases to add to the resident teaching file.
- Continue to serve as liaison between BCC and the mammography department. The latter is often not informed on decisions and projects taking place in BCC.
- Continue to identify very high risk patients who may be interested in the BRCA gene education and screening program now offered by BCC. These names are forwarded to BCC for future contact.
- Participate in the regularly scheduled BCC research meetings as to ongoing and potential projects involving BCC.
- Established contact with Dr. Edison Liu, Director of the Division of Clinical Sciences of the National Cancer Institute (NCI) for future collaborations.
- Attended the first Breast Cancer Think Tank sponsored by the NCI to foster collaboration between clinicians and laboratory researchers in their quest for new treatments and cures for breast cancer.
- Attended conference on the Frontiers in Breast Cancer Research.
- Attended the annual meeting of the Radiological Society of North America (RSNA), which is the world's largest and premiere radiology meeting. I attended presentations of new papers and studied exhibits of breast cancer research. Breast cancer was a very popular topic this year. Made new and reinforced old contacts with colleagues in mammography and related breast imaging fields.

- Begin to refer patients with complex problems to MRI and to nuclear medicine for scintimammography. The latter is separate and different from the above described FDG protocol. Imaging of the breast now often requires multiple modalities, and this idea was reinforced at the above RSNA meeting.
- Begun as a reader for a digital mammography project run by Uniformed Services University of the Health Sciences (USUHS) and the University of South Florida.
- Attended a meeting at the Jackson Foundation to advise the Department of Defense (DoD) group on the writing of their proposal for the digital mammography and mammography mobile van project.

Goals/Objectives For Next Reporting Period

- Continue to provide coverage in the mammography section of the radiology department.
- Defend the above-mentioned proposal to the IRB.
- Funding for digital acquisition mammography equipment has been approved for CAPT Jerry Thomas of USUHS. NNMC will one of the test sites for General Electric's digital acquisition mammography unit. Projects will have to be designed in collaboration with other testing sites.
- Meet with the members of the Transfer of Intelligence Technologies to Improve Breast Cancer Imaging Project (TITIBCI) regarding preliminary data now being collected.
- Begin stereotactic breast biopsy procedures using the mammotome, a new device recently purchased by BCC for the mammography department.
- Continue dialogue with radiologists at NIH (National Institutes of Health) for possible future imaging of our patients on their positron emission tomography (PET) scanner, as well as possible biopsies of our breast patients on their Magnetic Resonance Imaging (MRI) scanner.
- Continue dialogue with researchers at NCI and NIH regarding a nutritional epidemiological study on breast cancer patients at NNMC. A proposal will be written and submitted.

MCINTYRE

Description Of Work To Be Performed

- Support a research program which focuses on breast cancer.
- Liaison between the Radiology Department-Mammography Section, the Breast Care Center

(BCC), and other hospital departments.
- Perform nursing duties.
- Perform managerial duties.

Technical Objectives For The Reporting Period

- Assist the Radiologists/staff with stereotactic and ultrasound guided breast biopsy procedures.
- Perform assessments on all stereotactic/ultrasound biopsy patients and provide these patients with post breast biopsy teaching instructions.
- Assist with continued development between the BCC and Radiology Department, as the patient volume increases.

Summary Of Work Performed During Current Reporting Period

- The above technical objectives were met during the current reporting period.
- Assisted with the re-organization of the mammography scheduling process.
- Supervised other mammography personnel.
- Completed statistics for FDA/ACR mammography inspection for 6/96-5/97 period.
- Tracked 6 month follow-up patients with outcome analysis via BCC Task Management Tool.
- Assisted with and completed the BCC "Nurse Case Manager (NCM) curriculum" for 7/97-9/97 period.
- Assisted the BCC with "Educate the Educator" program in the Radiology Department-Mammography section.
- Correlated mammography and pathology findings via CHCS.
- Assisted the BCC with ambulatory/nurse case manager interviews.
- Assisted the new incoming mammography file clerk with his position.
- Assisted newly promoted mammography scheduler with her position transition.
- Attended a 3 day conference in Region #5 in 11/97.
- Was promoted from NC M with the BCC to Program Manager with the Biomedical division. Will transition to this position 1/98.

Goals/Objectives For The Next Reporting Period

- Continue to perform nursing and managerial duties, as described above, until I transition to my new position 1/12/98, in Rockville, MD.
- Continue to obtain mammography statistical data for FDA purposes on a monthly basis.
- Track 6 month follow-up patients with outcome analysis via BCC Task Management Tool.
- Attend nursing/management conferences when available.
- 12/97- Assist the new radiology nurse case manager with her transition to my previous job position. 1/98- Start my new job position as Program Manager with the Rockville, MD, office. Will encourage the military regions to use GEO-CENTERS for staffing purposes, in reaching their BRIN objectives.

O'HALLORAN

Description Of Work To Be Performed

- Collaborates with a multidisciplinary staff concerning patient needs and identifies patients who may benefit from services such as social service, physical therapy or nurse case management.
- Performs professional nursing assessments
- Opening and closing all clinical areas and preparing exam rooms for patient use
- Triage of telephone calls and patient walk-ins
- Responsible for all clinical functions
- Acts as Relief Clinical Nurse Manager, in the absence of the Nurse Manager
- Carries out of physician's orders
- Reviews and sorts pathology and mammogram reports
- Oversees preparation of charts for patient visits
- Assignment of nursing lunch breaks to ensure appropriate coverage of the unit
- Processes linen and hazardous material
- Maintains supplies for clinical exam rooms and needle/syringe cart
- Coordinates all FNAs and procedures and notifies Nurse Case Manager of positive diagnosis
- Attends seminars/conferences
- Performs biopsy teaching and APU coverage in absence of Clinical Nurse Educator

Technical Objectives For The Reporting Period

- Organization of patient charts
- Maintain mammography scheduling book
- Modification of daily clinic schedules
- Utilization of Care Central for tracking of FNAs
- Continue to improve computer skills
- Identify nursing roles for ambulatory care setting
- Development of orientation for new nursing staff
- Stocking of all clinical areas
- Breast self examination teaching
- Organization of triage area and triage files

Summary Of Work Performed During Current Reporting Period

- Participated in multidisciplinary meetings to further enhance the relationship between BCC, SSU and GSC
- Enhance nursing knowledge base on breast cancer issues
- Further developed personal computer skills
- Triage telephone calls and walk-ins
- Further identified the nursing assignments of the ambulatory care staff
- Coordinated all FNAs and procedures and notify Nurse Case Manager if positive
- Continued to orient new staff members to patient flow processes and forms within the BCC
- Coordinated patient flow activities in the clinical areas with patients, nurses and physicians

Goals/Objectives For Next Reporting Period

- Learn the role of Radiology Nurse Case Manager
- Continue to attend courses towards Masters of Science in Nursing degree
- Become familiar with administrative functions and their affect on nursing role
- Work towards integration of physicians, nurses and other personnel to improve and support clinic functions.

RAPSON

Description Of Work To Be Performed

- Coordinate patient flow activities
- Perform professional nursing assessments
- Teach breast self examination
- Prepare patient charts with appropriate medical, lab ,and x-ray reports
- Assist physicians with all procedures such as FNA or cyst aspirations
- Provide physical and emotional support to patients during their appointment
- Collaborate with a multidisciplinary staff concerning patient needs and identifies patients who may benefit from services such as social service, physical therapy, or nurse case management
- Responsible for preparing all clinical areas for patients and securing clinical areas at the end of the day
- Process linen and hazardous wastes within the BCC
- Maintain supplies at par level and records supplies needed

Technical Objectives For The Reporting Period

- Continue development in the role of the ambulatory care nurse
- Continue development of computer skills, especially the use of hospital's system called CHCS
- Continues to gain further knowledge and education in breast cancer and it's treatment

Summary Of Work Performed During Current Reporting Period

- Coordinated patient flow activities
- Performed nursing assessments
- Provided BSE teaching
- Prepared patient charts appropriately with medical, lab, and x-ray reports
- Assisted physicians with many procedures done in the BCC
- Provided physical and emotional support to patients
- Collaborated with social service, nurse case manager, clinical nurse educator, physical

therapist and many physicians to ensure exceptional patient care
- Disposed of linens and hazardous wastes appropriately

Goals/Objectives For Next Reporting Period

- Continue to enhance education in breast cancer and its treatment
- Continue to improve patient flow management
- Continue to improve computer skills
- Attend a seminar/conference related to breast cancer
- Continue to participate in multidisciplinary meetings
- Continue to improve chart review

ROGERS

Description Of Work To Be Performed

- The social worker will interview and assess newly diagnosed breast cancer patients and provide them with educational materials, support group information, and a description of available social work services. The assessment will include a screening for depression and adjustment, the documentation of the patient's social history and a defining of the patient's environmental support systems.
- The social worker will evaluate and monitor the breast cancer patient's psychosocial and mental status and offer individual, couple, family, or group psychotherapeutic intervention or referral as appropriate.
- The worker will facilitate and encourage the identification of the patient's concrete needs and concerns and actively participate with the patient in a solution and task focused pursuit of such.
- Facilitation of the Stage I & Stage II Breast Cancer Survivor Group
- Facilitation of the Advanced Breast Cancer Support Group
- Facilitation of the Spouses of Breast Cancer Patients Support Group
- Solicit new member participation in the aforementioned Breast Care Center support groups.
- Collect and analyze the support group research data related to the Adjustment and Social Support in Male Spouses of Breast Cancer Patients.

- Act as the liaison between the Breast Care Center and the National Naval Medical Center Social Work Department. As such, the worker will attend all social work staff meetings, offer professional coverage and other services when necessary, coordinate communication, and maintain collegial rapport and interaction.
- Coordinate Breast Care Center patient participation in the American Cancer Society "Look Good, Feel Better" program for patients undergoing or having completed radiation and chemotherapy treatments.

Technical Objectives For The Reporting Period

- Address the psychosocial and concrete needs of individual patients in the Breast Care Center.
- Provide individual psychotherapy to patients experiencing significant emotional distress following diagnosis.
- Facilitate on-going therapy for patients who have experienced specific types of concerns at the completion of treatment including sexuality and intimacy issues, fear of recurrence, family concerns, etc...
- Facilitate psychotherapeutic intervention with couples who wish to enhance coping skills and increase the level of communication, sense of well-being, and stability in their union during a time of dramatic change and crisis following the diagnosis of breast cancer.
- Work toward beginning the American Cancer Society "I CAN COPE" program at the Breast Care Center. This program will serve to help breast cancer patients to communicate and network with other survivors throughout diagnosis, treatment, and beyond.
- Develop social work involvement with the BRCA Gene Study. Social Worker will serve as individual providing therapy to patients who experience anxiety, depression or other feelings related to the gene testing process and results of the tests. Examining the option of starting a genetic support group.

Summary Of Work Performed During Current Reporting Period

- Addressed the psychosocial status and patient/family concerns in the Breast Care Center.
- Worked closely with the Breast Care Center Nurse Case Managers to provide seamless care to patients. This included daily integration and discussion of services provided to ensure a continuity patient care and enhanced patient satisfaction.

- Provided facilitation of the Advanced Breast Cancer Support Group, the Stage I & Stage II Breast Cancer Survivor Group, and the Spouses of Breast Cancer Patients Support Group.
- Worked closely with the CHAMPUS and Supplemental Care offices to ensure that patients wig and breast prosthetics requirements were approved prior to the purchase of such items.
- Actively promoted the Breast Care Center Breast Cancer Survivor groups resulting in increased new membership
- Attended all National Naval Medical Center Social Work Department meetings, offered professional coverage, coordinated communication, and maintained constant collegial rapport and interaction.
- Became an active member of the Medical Ethics Committee of the National Naval Medical Center.
- Coordinated Breast Care Center patient participation in the American Cancer Society "Look Good, Feel Better" program.
- Compiled a comprehensive listing of wig salons for patients who are undergoing chemotherapy and may need to locate a wig prosthetic as a result of hair loss.
- Developed a comprehensive listing of local lodging with current prices and military or patient discount information for use by patients and their companions.
- Completed preliminary study and consideration of the American Cancer Society "I CAN COPE" program for possible implementation at the Breast Care Center.

Goals/Objectives For Next Reporting Period

- Continually provide comprehensive and high quality psychosocial and concrete services and interventions to Breast Care Center patients and their families.
- Coordinate and facilitate individual, couple, family and group psychotherapy for breast care patients.
- Continue to prepare and complete CHAMPUS and Supplemental Care documents for the procurement of necessary concrete items by breast care patients.
- Expand the BCC social work library to include more texts that address the emotional issues related to breast cancer.
- Continue working relationship with the American Cancer Society to bring to the Breast Care Center quality programs which address psychosocial issues related to cancer.

- Participate actively in the Educate the Educator Program sponsored by the Breast Care Center.
- Begin to develop a guided imagery meditation, stress reduction, and relaxation tape for use by breast care patients, their family members and loved ones.

SNEE

Description Of Work To Be Performed

- Case manages new breast cancer patients
- Utilizes the "Care Manager" software to document and track the patient's progress through the clinical care pathway of breast cancer treatment
- Helps to educate newly diagnosed breast cancer patients about disease, treatment, and follow up care
- Provides educational materials to patients and families
- Coordinates and plans appointments for multidisciplinary care in hospital, including, but not limited to hematology/oncology, radiation/oncology, plastic surgery, physical therapy, and social services
- Teaches patients about prosthetics and assists patient in preparing appropriate forms necessary to obtain prosthetic
- Provides emotional support to women and their families who are facing cancer treatment through verbal and nonverbal communication
- Provides support, comfort, and education to the patient through the use of pre and post op phone calls and by visiting the patient while they are an inpatient.
- Ensures that patients are receiving adequate follow up care
- Tracks breast biopsies and notifies doctor of any malignant pathology reports and ensures that patient is scheduled for appointment with physician
- Teaches and demonstrates the "Care Manager" software to interested personnel both within NNMC and at outside facilities
- Assists as needed in clinic as either ambulatory care nurse or nurse educator

Technical Objectives For The Reporting Period

- Ongoing development in the role of the nurse case manager

- Implement processes that will enable appropriate follow up care for breast cancer patients
- Continues to revise and perfect methods to discuss cancer diagnosis with patients
- Continues to gain further knowledge and education in breast cancer and its treatment
- Ongoing development of organizational skills to manage multiple patients and their individual needs
- Continues to provide "Care Manager" demonstrations to interested parties coming to the BCC

Summary Of Work Performed During Current Reporting Period

- Functioned as the sole nurse case manager in the BCC from mid-September and continues to do so at present after resignation of nurse case manager colleague
- Assisted in the interview process to identify candidates for an ambulatory care nurse and nurse case manager in the BCC
- Suggested and implemented useful changes in the care manager software
- Helped to educate patients and families on breast cancer
- Provided emotional support to women from diagnosis to completion of breast cancer treatment
- Collaborated with multiple disciplines to arrange for patient care
- Developed useful methods for managing many varied and complex patients
- Taught many new cancer patients about breast and wig prosthetics and assisted them in obtaining the prosthetics
- Attended tumor board meetings and was prepared to give additional information
- concerning breast cancer patients if required or requested by physicians
- Collaborated with staff on the development of a cancer database
- Provided education and working demonstration of the "Care Manager" software to interested personnel both within NNMC and to outside facilities
- Collaborated with the nursing staff to begin the redesign of the ambulatory nursing role

Goals/Objectives For Next Reporting Period

- Orient and train a new nurse case manager
- Work to establish guidelines for information entry in the "Care Manager" software

- Assist the ambulatory care staff with daily activities in the unit as the new nursing personnel are oriented and trained
- Assist the nursing team to redesign the nursing roles in the clinic, in particular, the roles of the ambulatory care nurses
- Continue to improve skills as a nurse case manager
- Assist in the development of a breast cancer database
- Continue to enhance my education in breast cancer and it's treatment
- Continue to improve computer skills
- Attend a seminar/conference related to breast cancer
- Continue to participate in multidisciplinary meetings
- Establish guidelines for case management follow up after the acute stage of diagnosis and treatment of the breast cancer patient
- Plan and develop, with nursing and medical personal, a form to be placed in the patient's chart that indicates that patient's individual recommended clinic follow-up schedule after she is diagnosed with breast cancer

SNYDER

Description Of Work To Be Performed

- Develop and integrate a breast care educational program for female/male Department of Defense beneficiaries and their support persons.
- Educational program to include all breast care issues with an emphasis on early detection of breast cancer.
- Provide pre-operative teaching and educate patients regarding breast cancer and treatment options.
- Being available as an information resource person for the patient and their support person.
- Plan staff development programs and maintain BCC staff development records.
- Act as relief Ambulatory Care Nurse under the direction of the nurse manager.
- BCC designated safety representative, responsible for safety manuals, monthly safety meetings and BCC safety issues.
- BCC representative on the Education Council Committee.
- BCC representative on the Nurse Practice Committee.

Technical Objectives For The Reporting Period

- Continue to provide patient education.
- Continue to develop array of patient educational materials.
- Continue to act as relief ambulatory care/ triage nurse.
- Continue staff development and safety representative responsibilities.

Summary Of Work Performed During Current Reporting Period

- Continued responsibility as the designated safety representative of the BCC.
- Participated in command sponsored health fairs.
- Maintained credentialing data base on all Geo-Center employees.
- Plans and institutes staff education calendar and events.
- Functions as Clinical Educator providing teaching on breast self examination, pre and post operative instruction and breast cancer.
- Functioned as relief ambulatory care/ triage nurse providing breast self exam teaching, assisting the physicians with physical exams, procedures, and scheduling of diagnostic tests when needed. Participated in health fair/wellness program.
- Continued to review educational materials and order needed materials.
- Preparation of the Genetics Education program for Physicians and Nurses.
- Preparation/ Implementation of the Orientation program for the new employee RN.
- Participation on the Educational Council Committee.
- Participation on the Nurse Practice Committee.

Goals/Objectives For Next Reporting Period

- Continue responsibility as safety representative.
- Continue to function as Clinical Nurse Educator providing teaching to patients and their support persons.
- Continue to function as relief Ambulatory Care Nurse.
- Identify needed materials and supplies for procurement.
- Participate in Wellness programs.

VAUGHN

Description Of Work To Be Performed

- Medical filing for the Radiology department and the Breast Care Center.
- Enter CHCS orders for comparison mammograms.
- Track mammogram films.
- Handle mail and telephone correspondence regarding radiology films.
- Pull and file mammograms.
- Make copies of mammogram films for physicians.

Technical Objective For The Reporting Period

- Alphabetize the main mammography file system.
- Systematic checking for quality improvement.
- Improve report filing to allow for more efficient operations.
- Being readily available for assistance to co-workers, the BCC staff, physicians and patients requiring assistance with mammography films.

Summary of Work Performed During Current Reporting Period

- Provided assistance to staff requesting help with mammography films.
- Continued to organize log book to improve film tracking.
- Continued to disseminate films to patients via CHCS computer.
- Assisted radiologists with research projects by providing mammogram films.
- Performed increased duties as patient volume increased within the BCC.
- Was promoted to the mammography scheduler in 10/97. Started to transition to this new position and continued with file clerk duties as described above.
- In 11/97, trained the incoming mammography file clerk.

Goals/Objectives For Next Reporting Period

- As I have transitioned to the permanent position of mammography scheduler, will become more proficient with the mammography scheduling process.
- Will work towards reducing the turnaround time for mammography studies.
- Continue preparation/ implementation of the Genetics Education program.
- Continue staff education calendar and events.
- Discussion with a BCC physician regarding preparation of an abstract about the Nurse Case Manager Curriculum.
- Continue to participate on the Education Council Committee.
- Continue to participate on the Nurse Practice Committee.
- Prepare a successful Orientation Program for the new RN employees.
- Acting Team Leader and Triage Nurse during the interim of hiring a full-time Ambulatory Nurse.

WALLACE

Description Of Work To Be Performed

- Act as Administrator of the Breast Care Center, responding to the needs of patients and staff to meet daily administrative requirements
- Oversee/Manage appointment scheduling system that allows for: maximum access of patients into the clinic, provides for medical training, research protocols, and administrative time, and is responsive to unanticipated demands and special cases.
- Gather workload data, prepare statistical reports, and analyze data to provide information and guidance.
- Patient ombudsman for the Center during Nurse Manager's absence.
- Coordinate input in order to prepare the annual budget, mid-year reviews, and unprogrammed requirements for the Center. Provide recommendations to the administrative team in the development and formulation of budget requests, based on familiarity and knowledge of Department programs and appropriate procedures, review and analyze budget requests, and determine whether requests for funds and expenditures are proper, necessary, and timely. Monitor use and rate of expenditures of budgeted funds. Oversee funding for all research conducted at the Center, with particular emphasis on clinical trials.

- Responsible for coordinating responses to all correspondence that comes into the Center. This includes Congressional inquiries, complaints, requests for information, requests for guest speakers, etc.
- Coordinate all reports generated in the Center. This includes establishment of a system that will guarantee reports are on time and that all reports reflect accurate data.
- Maintain oversight of equipment inventory and ensure that equipment is maintained.
- Review space utilization within the Center and advise the administrative team on such activities as space allocation and renovation.
- Supervise GEO-CENTERS, INC personnel located in the Breast Care Center, Building 10, 4th Floor, West..
- Manage information systems hardware and software within the Center. Primary coordinator for CHCS and ADS within the Center.
- Maintain oversight of the ordering process for supplies.
- Primary liaison between the military and GEO-CENTERS, INC.
- Provide advice on manpower utilization, work flow, and operational procedures.
- Respond to requests for administrative reports; generate, collate, synthesize and present a wide range of data in written or oral form; edit reports prepared by other members of the Department; and, confer with the administrative team in identifying and resolving administrative problems and needs.
- Coordinate staffing with the Nurse Manager, analyze manpower utilization and participate in interviews.
- Monitor legal issues. Make Staff Judge Advocate's office aware of potential litigation.
- Work with administrative team to develop plans for guiding future clinic operations.
- Oversee use of the Ambulatory Data System (ADS) for the Center.
- Assist Contract Management Department with maintaining accurate and complete files on contract employees.
- Assist in preparation for VIP tours and briefings.
- Other administrative functions as necessary.

Technical Objectives For The Reporting Period

- Oversee conversion of patient charting system. Ensure appropriateness of conversion plan. Develop timeline for conversion.
- Analyze current staffing and future staffing needs. Determine effectiveness of current nursing staff positions. Hire personnel as necessary. Orient new employees.

- Monitor Breast Cancer Prevention, Education, Diagnosis Initiative issues closely. This includes preparation of Statements of Work and proposals for obligation of future funding and completion/ submission of expenditure of funds reports to the Office of the Assistant Secretary of Defense and Tricare Region 1 Lead Agent's office.
- Further investigation of the integrity of data, with particular attention to use of CHCS, ADS and CareCentral Software.
- Continue to maintain compliance with the Surgeon General's ADS standards.
- Attend weekly meetings of the Information Management Quality Management Board to keep up-to-date on all information systems issues.
- Oversee procurement ordering process. Make sure all necessary supplies are ordered in a timely fashion. Ensure proper documentation.
- Participate in genetics research and cancer database development working groups.
- Act as Point of Contact and Coordinator of the John Silva/Defense Advanced Research Projects Agency project to develop an electronic patient file.
- Assess Ellora Software Inc. projects status. Monitor fund use versus project completion.

Summary Of Work Performed During Current Reporting Period

- Coordinated administrative activities of the BCC.
- Oversight of chart conversion.
- Assigned Assistant to the Administrator to assess accuracy of CHCS and ADS data entry.
- Managed schedule templates, discussed need to change templates with ambulatory care nurses.
- Maintained relationship with Contract Management Department.
- Participated in interviews for Nursing Staff. Hired one Patient Service Representative and three nurses.
- Coordinated with Budget Department to match records, provided budget for FY 98, coordinated memorandums in search of future funding.
- Handled administrative issues as necessary.
- Oversaw conversion of patient charting system. Ensure appropriateness of conversion plan. Develop timeline for conversion.
- Analyzed staffing needs to determine effectiveness of current staff positions. Hired personnel as necessary. Oriented new employees.

- Monitored Breast Cancer Prevention, Education, Diagnosis Initiative issues closely. This includes preparation of Statements of Work and proposals for obligation of future funding and completion/ submission of expenditure of funds reports to the Office of the Assistant Secretary of Defense and Tricare Region 1 Lead Agent's office.
- Attended weekly meetings of the Information Management Quality Management Board.
- Oversaw procurement ordering process. Made sure all necessary supplies were ordered in a timely fashion.
- Participated in genetics research and cancer database development working groups.
- Acted as Point of Contact and Coordinator of the John Silva/Defense Advanced Research Projects Agency project to develop an electronic patient file.
- Oriented new Nurse Manager
- Successfully passed Inspector General's Visit.

Goals/Objectives For Next Reporting Period

- Resolve remaining staffing issues. Hire Ambulatory Care Nurse
- Increase workload collection. Analyze new areas for data collection. Monitor physician participation.
- Increase knowledge of Tricare. Prepare the Breast Care Center staff for Tricare.
- Prepare for JACHO.
- Continue participation on genetics research and cancer database working groups.
- Continue to coordinate administrative activities of the BCC.
- Increase budget management, continue to actively seek new avenues of funding.
- Increase pressure on Ellora to provide project completion and expenditure of funds documentation.
- Monitor legal issues.
- Monitor compliance between BCC records and Budget Department records.
- Monitor procurement process more closely.
- Actively seek methods to improve current work practices.

II. NMRI, Bethesda, MD

F. BREAST CANCER RESEARCH & EDUCATION INITIATIVE (BRIN)

BELLITT, ELLIOTT, FREEMAN, FUSCHINO, HAMMA, MATTHEWS, SEMONES, STEWART, WHITEHEAD

Description Of Work To Be Performed

- The Department of Defense (DoD) has recognized and emphasized the importance of increased awareness and education regarding breast cancer and screenings. There are about two million DoD women beneficiaries over the age of 30, which represents 26% of all Military Health Services System (MHSS) beneficiaries. Thirteen percent of the active forces are women, and each year nearly 18,000 new cases of breast cancer are diagnosed in the MHSS. It is through education and awareness of the importance of clinical examinations, mammography, and monthly breast self-examinations (BSE) that breast cancer mortality can be decreased while positively affecting the morale of the DoD workforce.
- The FY97 BRIN Program utilizes a three-phased approach. Phase I focuses on beneficiary access to breast cancer screening, diagnosis, and treatment. Phase II will be implemented by the Military Treatment Facilities (MTFs), and focuses on training programs for all MTF Primary Care Managers on clinical breast cancer examinations and BSE techniques for beneficiaries. Phase III focuses on region-wide education programs.

Technical Objectives For The Reporting Period

Staff in Region V are responsible for:

- Enrolling patients in breast Care Programs.
- Developing pre and post care guidelines for stereotactic and ultrasound guided core biopsies.
- Increasing access by decreasing time between diagnosis of potential problem to mammogram or ultrasound guided core biopsy.
- Provide breast care education programs to the MTF community.
- Develop a Mail Education Reminder Program.

Summary Of Work Performed During Current Reporting Period

- Continue to provide high quality images for accurate radiologic interpretation.
- Maintained film processors within the set guidelines reducing departmental down time.
- Continue to mentor newer technologists by setting realistic goals and providing guidance.
- Decrease time to mammogram or ultrasound guided core biopsies from 5 months to in some cases, less than 24 hours.
- Set up a schedule and implemented the first wave of the Mail Education Reminder Program
- Held various breast care training sessions throughout the region.

Goals/Objectives For Next Reporting Period

- Continue to provide high quality exams in a timely manner with a continued emphasis on patient care and comfort.
- Strive to keep patient backlog with a two week time frame as opposed to the previous backlog of five months.
- Complete the second and third wave of the Mail Education Reminder Program.
- Continue training in the community.
- Continue to provide high quality, compassionate care to the military beneficiaries.

II. NMRI, Bethesda, MD

G. DIRECTED ENERGY EFFECTS RESEARCH

ELLIOTT

Description Of Work To Be Performed

- Design and implement a training program for Rhesus monkeys with ultimate goal of animals trained to perform visual acuity tasks while aligned on and being imaged by a Scanning Laser Ophthalmoscope.
- Select and screen candidates for above
- Install, operate, and maintain Rodenstock Scanning Laser Ophthalmoscope (SLO)

Technical Objectives For The Reporting Period

- Define Animal Performance criteria and Design Training program: The objective of this research is to evaluate the effects of Q-switched laser exposure on the visual performance and retinal morphology on Rhesus monkeys performing visual tasks while simultaneous retinal imaging with SLO takes place. The preliminary steps of this training program involve a sequence of interim training objectives.
- Selecting Subjects: Suitable Rhesus monkeys must be selected from a pool of available animals at BAFB. Candidates must be examined to determine general health, compatibility with training programs, and ophthalmological fitness.
- SLO Installation: Laboratory space must be located, identified, and coordinated with co-investigators for the SLO and the delivery and installation must be supervised.

Summary Of Work Performed During Current Reporting Period

- Training Program Design: A training protocol has been designed, equipment acquired and modified, and the initial stages of animal training begun with excellent progress. The computer program controlling the behavior modification system has been modified and refined.

- Subject Screening and Acquisition: Two of the Rhesus monkeys that were screened were found acceptable. They have been placed on the protocol and are in the initial stages of training.
- SLO Installation: The new Rodenstock SLO has been delivered and is in routine operation.

Publications, Abstracts, etc.

- ARVO Presentation May 14: Scanning Laser Measurements in Dorzolamide and Placebo Treated Eyes: Arteriovenous passage times in Large and Small Vessels During Hyper-and Hypo-Capnia. **W.R. Elliott**, D.L. Shipman, J.T. Kavanagh, W.E. Sponsel, J.Ness.

Goals/Objectives For Next Reporting Period

- Continue training subjects.
- Prepare new training apparatus to introduce the SLO into training system.
- Prepare SLO for mounting on optical bench.
- Modify behavior modification software to display stimuli on SLO raster.

GUILLORY

Description Of Work To Be Performed

- Compile and analyze data comparison of tactical in-flight information versus visual data collected via manual video tracking (spatial analysis).
- Support data collection at remote field sites for future analysis.
- Assist in any logistical support necessary.

Technical Objectives For The Reporting Period

- Reinforce information to pilots of the threat that hand held lasers pose to any flight operation. Create empirical parameters denoting laser hazard zones allowing the pilot tactical planning before a mission.

Summary Of Work Performed During Current Reporting Period

- Acquired spatial positioning data from actual flight sensor data comparison (data scoring).
- Provided logistical support during data acquisition mission to NSAWC, NAS Fallon, NV.
- Provided daily research and administrative support.

Goals/Objectives For Next Reporting Period

- Continue data scoring and logistics assistance.
- Learn more about laser technology and the impact on aviators.
- Provide additional logistical support for the continuing scientific mission.

RICHARDSON

Description Of Work To Be Performed

- Provide Biological Science Laboratory Technician (Animal) Support to the Microwave Department. Handling and training of non-human primates.
- Recording and compiling data.
- In-house management of non-human primates.
- Administrative support of animal use projects.

Technical Objectives For The Reporting Period

- At this time will be giving technical support to other technicians with their projects, and assist where needed in animal research. Recording data and compiling data on computer programs for publishing.

Summary Of Work Performed During Current Reporting Period

- Assigned duties as Lead Technician in the Millimeter Wave Eye Project.
- Attended and completed computer courses in order to abetter perform data collection and analysis duties.
- Responsible for animal vivarium, ensuring compliance with AALAC standards, animal health and welfare and functioning as the Detachment Liaison with Vet Sciences.

Goals/Objectives For Next Reporting Period

- Continued member of the Animal Use Committee.
- Continue data collection and analysis for Publishing.
- Continue supporting other technicians with various projects, as well as performing duties as lead technician on the Millimeter Wave project.
- Continue performing responsibilities regarding the Animal Vivarium.
- Continue computer training.

THOMPSON

Description Of Work To Be Performed

- Provide technical and analytical support for pulsed laser glare projects.
- Provide support in the experimental design and analytical support for visual psychophysical studies.

Technical Objectives For The Reporting Period

- Continue to coordinate the development of new laboratory facilities for continuation of Pulsed Light projects.
- Assist in research, design, and acquisition or various computer, laser, and stimulus generating software and hardware for new laboratory.
- Provide data analysis and write technical document summarizing completed Pulsed Laser Light study.

Summary Of Worked Performed During Current Reporting Period

- Provided research, design and acquisition of computer hardware and software for new experimental Pulsed Light study.
- Provided consultation in purchasing of electronic equipment for future glare study.
- Provided consultation in purchasing back projection screens for future glare study.
- Performed as liaison between client, BARCO and Pro-Line Video for development of stimulus interface.
- Developed hardware design for new veiling glare project.
- Provided statistical analysis, interpretation of results, and technical document for Pulsed Laser Light glare project.
- Continued to provide support in the development of the HUD and MFD psychostimulus displays.
- Began development of software/hardware interface using LabView software for new veiling glare study.
- Developed software/hardware interface for new veiling glare project.
- Interface was tested and performed successfully.
- Designed and installed bench top component topography for laser operation.
- Installed and successfully tested and ran demonstrations of new 5W Coherent laser for veiling glare project.

Goals/Objectives For Next Reporting Period

- Complete development of second laser laboratory.
- Continue development of the experimental workstation and design a method for simulating aircraft cockpit instrumentation symbology.
- Develop experimental methodology for Phase III of laser glare project.
- Continue to provide statistical support for all laser department projects.

MARES

Description Of Work To Be Performed

- Function as Supply Technician for the Detachment, researching, processing and receiving of orders. Assist other supply technicians within the Detachment in order submission. Complete follow-ups on accounts on all purchases through the base supply departments ie. Medical Supply, Base supply and the Base service store account. Manage the Plant Property Program on monitoring the major and minor equipment inventory. Assist the Administrative Officer on drafting correspondence between the local supply department and the Detachment. Act as command secretary in the absence of the secretary.

Technical Objectives For The Reporting Period

- Will be processing orders for the Detachment.
- Update purchase orders that are past due.
- Do the monetary budget for base, medical and the base service store.
- Monitor the major and minor equipment inventory.
- Assist the Administrative Officer on drafting correspondence between the local supply department and the detachment.
- Act as command secretary in the absence of the Secretary.

Summary Of Work Performed During Current Reporting Period

- All objectives were met.

Goals/ Objectives For Next Reporting Period

- Continue Detachment Supply support.
- Become more educated on computer software programs related to my job.
- Continue education in Administration Functions.

III. NDRI, Great Lakes, IL and NDRI Detachment, Bethesda, MD

A. DENTAL DISEASES-RELATED RESEARCH

BECK

Description Of Work To Be Performed

- Provide technical assistance with ongoing research projects. Participate in National Institute of Dental Research (NIDR) linkage analysis projects. Maintain and upgrade the laboratory such that the research experiments are carried out smoothly. Maintain and record proper technical procedures and data produced for each experiment.

Technical Objective For The Reporting Period

- Assist Molecular Epidemiology of NIDR with linkage analysis studies of genetic disorders. Optimize polymerase chain reaction (PCR) conditions for bone morphogenic protein receptor (BMPR) primers such that common PCR conditions can be applied to all sample types.

Summary Of Work Performed During Current Reporting Period

- Continued to participate in NIDR Molecular and Epidemiology experiments. These studies deal with the inherited genetic disorders. Hundreds of DNA's are gathered from various sites and organized for the genetic analysis. Techniques of PCR and gel electrophoresis are used to investigate gene(s), candidate regions, responsible for the genetic disorder (Ex. cleft lip) being studied. In addition to candidate regions, the entire human genome region is being scanned for a potential marker linkage. This is an ongoing project.
- Significant progress have been made to optimize the primers and PCR conditions for measuring BMPR activities at the nuclear level. It will require more fine tuning of protocol before the similar procedure can be applied to various tissue types.
- Continue growing and maintaining various fibroblast cell lines for the purpose of identifying specific RNA messages by the *in situ* hybridization method. *In situ* hybridization method is useful when looking for a specific gene activation via mRNA production within individual cells of a tissue.

Goals/Objectives For Next Reporting Period

- Main objective of this upcoming quarter is to continue with NIDR Epidemiology projects.
- Continue optimizing the PCR conditions for the bone morphogenic protein receptor primers.
- Continue growing fibroblasts.

JONES

Description Of Work To Be Performed

- Senior Research Scientist. Responsible for the Molecular Biological and Molecular Genetic aspects of the projects. This includes the development, evaluation and refinement of molecular biological research protocols.

Technical Objectives For The Reporting Period

- Relative to the program entitled "Biomarkers for Oral cancer" for the Puerto Rico study subgroup, it is anticipated that the "first pass" SSCP analysis of p53 exons 7 and 9 of the p53 gene will soon be completed and that DNA sequence analysis will begin.
- Clearance for the expanded genetic analysis of the case-control samples from the Puerto Rico Oral Cancer Study was obtained. Will begin characterization of a number of polymorphic risk-associated genes for these samples.
- As additional patient-derived samples become available, anticipate expanded involvement in the NNDC Resident projects evaluating the role of bone morphogenetic protein (BMP) receptors in bone regeneration in periodontal tissues.
- Anticipate the continued arrival of specimens from the various sites participating in the VA subgroup of the Biomarkers for Oral cancer study. Will begin the isolation of DNAs from these materials and the analysis of genetic variation.
- DNAs from the Taiwan Nasopharyngeal Carcinoma Study subgroup will be further characterized using additional genetic markers. The arrival of additional DNA samples for this study is anticipated and these will be incorporated into the ongoing studies.

- Will characterize the DNAs from the Taiwan Oral Cancer Case - Control Study subgroup to determine the frequencies of additional polymorphic genes--especially those associated with high-risk behavior.

Summary Of Work Performed During Current Reporting Period

- Relative to the program entitled "Biomarkers for Oral Cancer" for the Puerto Rico study subgroup, "first pass" SSCP analysis of exons 5, 6 and 8 of the p53 gene has been completed. "Extended" PCR primers for each of the p53 exons under investigation were designed and the reactions incorporating them are now being optimized. These "extended" primers incorporate M13 sequences that will facilitate PCR-mediated bi-directional sequence analysis for mutation assessment. Numerous potential mutants have been isolated and are awaiting characterization via DNA sequence analysis.
- Relative to the program entitled "Biomarkers for Oral Cancer", for the Puerto Rico study subgroup, a large number of blood samples have been received in order to increase the number of cases and controls. A pilot test for the isolation of genomic DNA from these samples has been completed successfully.
- Relative to the program entitled "Biomarkers for Oral cancer," four shipments of DNA for the Taiwan Nasopharyngeal Carcinoma Study have been received. The information relevant to these DNAs has been compiled and is being readied for incorporation into a database in preparation for a large-scale study to identify genes associated with risk for NPC.
- A characterization of the association of several additional polymorphic genetic markers with smoking and other "risk-taking" behaviors for the Taiwan Oral Cancer Study was initiated. Significant differences in the frequencies of alleles of one form of the dopamine receptor have been observed between the oral cancer cases and healthy controls.
- Relative to the program entitled "Biomarkers for Oral Cancer," clearance for the initiation of the study of oral cancer in Greece was obtained. All blood samples available for this study have been processed and DNA isolated. A characterization of the frequency of polymorphisms within the CYP1A1 gene in the Greek population was carried out and the data have been included as part of an abstract submitted for presentation at the annual meeting of the American Association for Dental Research. Further characterization of these DNAs for polymorphisms within a set genetic markers associated with increased cancer risk is in progress.

- Relative to the program entitled "Biomarkers for Oral Cancer", samples continue to arrive for the VA Oral Cancer Study subgroup and are presently being archived. For tracking purposes a database of all samples received to date was created. Information on new samples will be added as they arrive.
- Have undertaken greater involvement in the NNDC Resident projects evaluating the role of bone morphogenetic protein (BMP) receptors in bone regeneration in periodontal tissues. Tissue samples for this study are arriving.
- Participated in the design of the Laboratory Information Management System (LIMS) database for all aspects of laboratory data management. A master database of all samples received to date for the Taiwan nasopharyngeal Carconoma Study was created and will be incorporated into the LIMS.

Publications, Abstracts, etc.

- A. Zavras, J.E. Jones, Y-F Wang, C.W. Douglas and S.R. Diehl. Molecular Epidemiological Investigation of the Etiology of Oral Cancer. (Abstract accepted) Talk to be presented at the 1998 AADR Annual Meeting in Minneapolis.

Goals/Objectives For Next Reporting Period

- Relative to the program entitled "Biomarkers for Oral Cancer" for the Puerto Rico study subgroup, it is anticipated that SSCP analysis of p53 exons 7 and9 of the p53 gene will be completed and that DNA sequence analysis using the extended primers will begin.
- Will begin large-scale isolation of DNA from the blood samples received for the Puerto Rico oral cancer case-control study. Will begin the characterization of a number of polymorphic risk-associated genes for these samples.
- Anticipate the continued arrival of specimens from the various sites participating in the VA subgroup of the Biomarkers for Oral cancer study. A tracking database has been established for these samples. Will begin the extraction of DNAs from these materials and the analysis of genetic variation.
- DNAs from the Taiwan nasopharyngeal Carcinoma Study subgroup will be further characterized using additional genetic markers. A master database of all samples now in hand has been established. Will begin the preparation of these samples for who-genome mapping in an attempt to identify the gene(s) associated with increased risk for masopharyngeal carcinoma. The arrival of additional DNA samples for this study is anticipated and these will be incorporated into the ongoing studies.

- Will further characterize the DNAs from the Taiwan Oral Cancer Study subgroup to determine the frequencies of additional polymorphic genes -- with special emphasis on those associated with high-risk behavior.

MILLER

Description Of Work To Be Performed

- Senior Research Scientist and Group Supervisor. Responsible for all aspects of Immunological, Microbiological, and Tumor Biomarker activities within the Naval Dental School. This includes the development and supervision of research protocols, dental resident mentoring activities, instruction of courses in dental microbiology and dental immunology, serving as a link between NIH sponsored research and Naval Dental Research programs, and troubleshooting of research programs, computers, instrumentation and equipment.

Technical Objectives For The Reporting Period

- Relative to the program entitled "Biomarkers for Oral Cancer," HPV evaluations on DNA isolated from a group of 150 tissue sections obtained from subjects from Puerto Rico will be completed and evaluated against epidemiological data including smoking incidence. It is anticipated that during the next quarter work will be initiated to evaluate HLA polymorphisms in this group.
- Relative to the project "Changes in Immunoglobulins as a Result of Smoking Cessation and Relation to Neurotransmitter Genes" funded by NIDR/NIH and jointly conducted by the Navy, Geo-Centers, NIDR, and individuals at the Jerry L. Pettis VA Medical Center in Loma Linda, CA., final blood samples will be collected and evaluated. It is anticipated that all samples will have been collected by the end of 1977. In addition, DNA will be extracted from blood samples in order to begin evaluation of dopamine receptor and transporter gene polymorphisms.
- Relative to the project "Characterization of Bone Morphogenetic Protein Receptors in Oral Tissues" evaluation of clinical samples will begin. This will involve the preparation of cDNA from RNA isolated from the tissue samples and completion of optimization of PCR conditions for remaining primer pairs.

- Relative to the project concerning the evaluation of cytokine production by oral fibroblasts, optimization of PCR conditions will continue and procedures for *in-situ* hybridization will be developed.
- A new project concerned with a survey of virus associated with periodontal and periradicular infections will be initiated. PCR based procedures will be used to identify specific viral types. During the next quarter it is anticipated that PCR conditions will be optimized for several viral DNA samples and that evaluation of some of the clinical samples will have commenced.
- It is anticipated that the course "Oral Microbiology" will be completed and that "Oral Immunology " will begin.

Summary Of Work Performed During Current Reporting Period

- Relative to the program entitled "Biomarkers for Oral Cancer," DNA isolated from a group of 150 tissue sections obtained from subjects from Puerto Rico has been tested for a variety of HPV genes using PCR procedures. Scoring of ABI 373 SDS polyacrylamide gels and dot-blot hybridizations have been completed. Tissue samples from our Greece study have been blocked in paraffin sectioned in preparation for DNA isolation. Finally, NIH funding has been obtained to permit the placement of two additional positions in our Biomarkers Group and the search process has begun.
- Relative to the project "Changes in Immunoglobulins as a Result of Smoking Cessation and Relation to Neurotransmitter Genes" funded by NIDR/NIH and jointly conducted by the Navy, Geo-Centers, NIDR, and individuals at the Jerry L. Pettis VA Medical Center in Loma Linda, CA., results from the initial phases of this study have been accepted for presentation ('Effect of Smoking Cessation on Total Serum IgG2") at the 1998 Annual Meeting of the American Association for Dental Research. To date, 66 subjects have been analyzed. We have found that IgG2 levels significantly decreased in the 24 subjects who had completely reduced their smoking or had cut back but not quit entirely.
- Relative to the project concerning the evaluation of cytokine production by oral fibroblasts, preliminary evaluation of reference markers (actin and cyclophyline) has been completed. In addition, a variety of cDNA's have been obtained from RNA isolated from gingival fibroblasts, pulpal fibroblasts and endothelial cells stimulated in culture with a variety of stimulators (TNF, growth factors, and bacterial components).

- Relative to the project "Characterization of Bone Morphogenetic Protein Receptors in Oral Tissues", initial clinical samples have been collected.
- A proposal to evaluate hypersensitivity and cytotoxic effects of soft denture liners has been approved by the NNMC Institutional Review Board and Human Use Committee. Initial studies involving the direct influence of Viscogel (Dentsply), Coe Comfort (GC), FITT (Kerr), Lynal (Caulk/Dentsply), and Coe Soft (Coe Lab) on fibroblast growth have been completed and culture fluids for cytokine evaluation have been secured.
- An updated text has been completed for use in teaching Oral Immunology to Dental Residents and the course has begun.

Publications, Abstracts, etc.

Publications:

- Euler, G, M.M. D'Alesandro, Hutter, J., and Miller, G. Interleukin -6 in neutrophils from peripheral blood and inflammatory periradicular tissues . Accepted for publication in The Journal of Endodontics.

Abstracts and Presentations:

- Euler, G., Miller, G., Hutter, J., and M.M. D'Alesandro. 1997 Interleukin -6 in neutrophils from peripheral blood and inflammatory periradicular tissues . Abstract No. 1242. Journal of Dental Research 76: (Abstract).
- Cummings,G., Lenoci, J.L., Malik, N.S., D'Alesandro, M.M. and G.A. Miller. 1997 Antibacterial effectiveness of temporary endodontic filling materials. Abstract No. 2969. Journal of Dental Research 76:(Abstract).
- Sonnier,K., D'Alesandro, M.M. and G.A. Miller. 1997 Association of superantigens in the development of advanced periodontitis. Abstract No. 3176. Journal of Dental Research 76: (Abstract).
- Miller, G.A. , Gu, X. , and S.R. Diehl. 1997. Comparison of gel and hybridization methods for detecting and subtyping human papillomaviruses in oral lesions. Proceedings of the American Association for Cancer Research 38: (Abstract).

Speeches:

- Invited seminar on the topic of cancer genetics, Epidemiology and Disease Prevention Branch, NIDR/NIH. February, 1997.

Funding

- Successfully competed for IR funding with Dr. M.M. D'Alesandro, MSC, USN with submission of the project "Protein pattern recognition for risk assessment of periodontal disease". Funding was approved as work unit number 0601152N 00004.001.0701.

Goals/Objectives For Next Reporting Period

- Relative to the program entitled "Biomarkers for Oral Cancer," the finial HPV control study to determine assay sensitivities will be completed. Data from the Puerto Rico DNA samples will then be evaluated against epidemiological data including smoking incidence. It is anticipated that during the next quarter work will be initiated to evaluate HLA polymorphisms in this group as well as to prepare a manuscript draft. In addition, it is also anticipated that selections will be made to fill the two new positions associated with our tumor biomarkers efforts.
- Relative to the project "Changes in Immunoglobulins as a Result of Smoking Cessation and Relation to Neurotransmitter Genes" funded by NIDR/NIH and jointly conducted by the Navy, Geo-Centers, NIDR, and individuals at the Jerry L. Pettis VA Medical Center in Loma Linda, CA., final blood samples (for a total of 200 subjects) will be collected and evaluated. It is anticipated that all samples will have been collected during the first quarter of 1998. In addition, DNA will continue to be extracted from blood samples in order to begin evaluation of dopamine receptor and transporter gene polymorphisms. In addition, efforts will be made to evaluate cotinine levels in all serum samples. These measurements will help to insure that subjects will be properly identified as to their smoking status.
- Relative to the project "Characterization of Bone Morphogenetic Protein Receptors in Oral Tissues" collection of clinical samples will proceed in preparation for assay.
- Relative to the project concerning the evaluation of cytokine production by oral fibroblasts, optimization of PCR conditions will continue and procedures for *in-situ* hybridization will be developed.
- A new project concerned with a survey of virus associated with periodontal and periradicular infections will be initiated. PCR based procedures will be used to identify specific viral types. During the first quarter of 1978 it is anticipated that PCR conditions will be optimized for several viral DNA samples and that evaluation of some of the clinical samples will have commenced.
- It is anticipated that the course "Oral Immunology" will begin and completed during the first quarter of 1998.

IV. NMRI TOX/DET Dayton, OH

A. TOXICOLOGICAL STUDIES

ADEMUJOHN

Description Of Work To Be Performed

- The purpose of the neurobehavioral laboratory coordinator at NMRI/TD is to provide technical support to various aspects of ongoing on-site projects in neurobehavioral research. During this quarter the coordinator has been and will be involved in neurobehavioral testing for the effects of simulated stress factors relating to the Gulf War Syndrome on animal models via computer-aided qualitative and quantitative methods. The coordinator also supervises animal training protocols for upcoming pharmaceutical exposure studies.

Technical Objectives For The Reporting Period

The major technical objectives for this quarter is as follows:

- Rangefinding using operant - trained animals and measuring subsequent stages of diminished capacity.
- To compile, catalog and computerize the above mentioned data.
- To train pigeons and rats for problem solving protocols
- To start and complete a drug dose curve on Diphenylhydantoin (DPH) on rats
- To compile and analyze previously collected data from the D-Amphetamine, Diazepam and Haloperidol studies completed.
- To obtain operant testing and training data for animals used in operant exposure testing .
- To organize, catalog and generate computer graphics, cumulatively from the above mentioned data.
- To maintain data for future reference in upcoming publications.
- To be responsible for the procurement and securing of all materials used in testing and training protocols
- Responsible for documenting and maintaining operant weights
- Responsible for writing and procurement of standard operating procedures for pigeon, rabbit and rat training protocols

- Responsible for overseeing daily accurate and detailed entries and updates of all work unit laboratory books.
- Responsible for maintaining quality control assurance for all ongoing experiments and/or protocols with / between work unit P. I.'s and laboratory technicians.

Summary Of Work Performed During Current Reporting Period

- Trained and conditioned new and incoming rodent and pigeon groups to protocol adaptation.
- Maintenance of all laboratory work unit notebooks
- Implemented several data methods to compile training data and weight maintenance on the all operants.
- Compiled stock animal drug history logs
- Compiling meeting memorandums for the OIC
- Trained all incoming personnel on standard procedures for lab techniques.
- Trained personnel (in house class) on GLP standards for recording raw data into lab notebooks
- Reviewed and edited S.O.P.'s on EEG, Porsolt, new pigeon and rat protocols.

Presentations :

- Ritchie, G.D., Rossi III, J., Hulme, M.B., Ademujohn, C.Y. and Cassell, J. Effects of $GABA_B$ antagonist CGP-35348 and human anti-epileptic drugs on spontaneous and chemically induced abscence-like SDW's in Fischer-344 rats. *Abstract*, Society for Neuroscience Annual Meeting, New Orleans, LA, Oct 1997.
- Ritchie, GD, Rossi III, J, Nordholm, AF, Hulme, MB, Ademujohn, CY, and Cassell, J. Effects ofGABA B antagonist CGP-35348 and human anti-epileptic drugs on spontaneous and chemically—induced abscence-like SDW's in Fischer-344 rats. *Society for Neuroscience Abstracts,* 1997; 23 (2): 940.6
- Ritchie, GD, Ademujohn, CY, MacInturf, S, Hulme, ME, McCool, C, Nordholm, AF, Rossi III, J, MacMahon, K, Leahy, H, and Wolfe, RE. Repeated exposure of Rats to JP-4 Vapor Induces Changes in Neurobehavioral Capacity and 5-HT/5-HIAA Levels. *J Toxicol Environ Health* Submitted for publication, Dec 1997.
- Same as above, was submitted as an abstract to the Navy Environmental Health Center Annual Conference, March 1998.

Goals/Objectives For Next Reporting Period

- To accurately oversee the training of rodents for various testing protocols, such as EEG, swimtest.
- To oversee rabbit testing, training and conditioning for upcoming neurobehavioral studies.
- To maintain a clean and orderly laboratory environment.
- To provide technical support in testing relative toxicity of various pharmaceuticals in pigeons and rats and rabbits.
- To procure and document pigeon maintenance pertaining to preparatory requirements for 'shaping' activities , pre-testing and testing protocols.
- Maintain quality assurance in all levels of data acquisition, processing and retrieval for all completed and ongoing lab experiments and protocols.
- To compile and organize the raw laboratory data into a centralized GLP standard retrieval system
- To start and complete drug dose curve/ study on haloperidol, diphenylhidantoin, and scopolamine.
- To publish results of above mentioned drug studies.
- To begin testing operants on schedule - induced polydypsia studies on Wistar rats.

BRIGGS

Description Of Work To Be Performed

- Dr. Briggs is the General Manager and Senior Contractor Representative for GEO-CENTERS, INC., for the NMRI contract at the Toxicology Detachment (NMRI/TD). He serves as a member of the Executive Steering Committee and performs toxicology research as an Associate Investigator. He is responsible for collaborating GEO-CENTERS, INC. resources in support of the toxicology research in support of the NMRI/TD mission. Dr. Briggs functions in response to taskings from the Officer-In-Charge of the Detachment. These duties include assuring compliance with the Quality Management Plan.

Technical Objective For The Reporting Period

- Correlate Geo-Centers, Inc resources with NMRI/TD in compliance with the 5450 Instruction and strategic planning and operational guidelines instituted by the Executive Steering Committee and the Officer in Charge and the Senior Scientist
- Coordinate in the transfer of neurobehavioral group research activities to the Veterans Administration Hospital in Dayton, Ohio
- Continue to establish capabilities to perform reproductive toxicology and dermal toxicology screening studies and validate the methods
- Initiate the DBNP acute oral, dermal and inhalation studies in rats
- Continue to integrate procedures and plans into the Quality Management Program. This includes the evaluation and implementation of a respiratory protection program, administrative procedures SOPs and audits of current and recently completed projects
- Prepare the Quality Management article and submit it for review and publication and performed two data audits and reviewed SOPs for administrative and QA functions.
- Prepare a poster for the regional Society of Toxicology Meeting and prepare abstracts for the Spring Conference and NEHC Meeting

Summary Of Work Performed During Current Reporting Period

- Helped to mentor the Executive Administrator and introduce him to procedures and methods for conducting toxicology research, marketing and military relevance issues. This included preparing 2 proposals for potential funding from the DoD/VA neurotoxicity program relating to stress-induced illnesses and assisted in the review of 6 other proposals. Prepared 3 pre-proposals for Program 8 funding and reviewed and prioritized 15 pre-proposals prepared by others.
- Assisted in the preparation of briefings by the OIC on DBNP, benzene and endocrine disruptors and for the project review meetings presented to NMRI, NHRC, DoD and the Army.
- Assisted the XA with responses for toxicity and exposure issues relating to HAN, potential reproductive toxicants, paint products and other chemicals of interest to the Navy
- Provided technical input into finalizing the protocols for conducting DBNP acute oral, dermal and inhalation studies. Two oral studies were completed, dermal studies are to be initiated early next quarter, and inhalation procedures could not be developed by the Air Force, so they will be developed by the Navy and performed next quarter

- Continued to finalize Standard Operating procedures as part of the Quality Management Program. Finalized a publication for a peer reviewed journal and submitted it for clearance
- Helped to finalize the NMRI/TD brochure

Publications:

- Submitted the final article for clearance and publication entitled The Establishment of Good Laboratory Practices at the Naval Medical Research Institute
- Presented the Poster entitled Assessment of Estrogens in the Naval Environment at the regional Society of Toxicology Meeting

Goals/Objectives For Next Reporting Period

- Assist with planning and technical support for toxicology studies conducted at NMRI/TD
- Continue to finalize Standard Operating Procedures and get them approved by NMRI/TD management Perform data audits and review technical reports as tasked by the O.I.C.
- Perform reproductive and dermal toxicological evaluations on chemicals of military interest. Get the new sperm analyzer ordered and complete Standard Operating Procedures for reproductive toxicology methods
- Assist and support research on the acute oral, dermal and inhalation studies with DBNP. At least one additional acute oral study and a dermal penetration study will be performed. Methods development for inhalation studies will be developed.
- Establish the respiratory protection program and ensure the proper training and documentation of the procedures

CONNOLLY

Description Of Work To Be Performed

- Cataloging print and non-print materials for circulation
- Ordering and maintaining serials collection, including claiming missing issues
- Handling reference questions
- Providing interlibrary loan assistance
- Locating needed materials in other libraries
- Preparing book orders

Technical Objectives For The Reporting Period

- Catalog materials as received
- Catalog materials not yet cataloged
- Provide library service to the toxicology community at WPAFB
- Continue working on a manual card catalog
- Become familiar with Microsoft Access database program
- Set up card catalog in Access
- Conduct an audit of library holdings
- Update journal holdings list

Summary Of Work Performed During Current Reporting Period

- Due to computer upgrades at this workstation, the database for the card catalog is no longer functional. The data were captured into Microsoft Access by the ADP department.
- Conducted an audit of 20% of monograph holdings. Less than 1% of the 800+ monographs were unaccounted for.
- Updated journal holdings list to accurately reflect each issue held by the library
- Provided updated journal holding list to the ADP department for posting on our new web site
- Obtained 39 journal issues to fill "holes" in our holdings, from duplicates held by other libraries. The only cost is reimbursement of postage at the library rate.
- 35 articles obtained from local libraries
- 4 books borrowed from local libraries for customers here
- 5 interlibrary loans obtained
- 3 interlibrary loans provided to another library
- 8 literature searches conducted using in-house CD/ROM database capabilities
- 7 searches successfully conducted on the Internet for customers, including downloading of documents as required
- 12 reference questions answered
- 7 telephone inquiries on journal locations in local area handled successfully
- 9 requests for articles located and filled from in house resources
- 6 articles obtained using the CARL UnCover system via the Internet
- 2 orientation/training session conducted
- 79 journal volumes consulted by customers

Goals/Objectives For Next Reporting Period

- Continue cataloging
- Continue preparing cards for the manual card catalog
- Continue training program
- Attend training on Microsoft Access
- Set up forms, reports, etc. for the Microsoft Access database now being used for the card catalog

HORTON, DIBLEY

Description Of Work To Be Performed

- Maintain Local Area Network (LAN)
- Maintain and upgrade individual Desktop and Laboratory Computers
- Provide answers, support and expertise in correcting computer problems, including all peripherals attached to these systems
- Continue comprehensive program for maintaining system integration and reliability through back-up procedures, documentation, and redundant systems
- Continue to update information Databases IRIS, Medline, Toxline and Serline
- Organize Media, Manuals and Spare Parts
- Prepare ASDPs for procurement of new computer systems, software and peripherals
- Maintain in-house software and databases

Technical Objectives For The Reporting Period

- Plan for and implement beginning stages for addition of VA to our LAN
- Modify the ADP SOP manual as needed
- Develop method for on-line user SOP via our Exchange system

Summary Of Work Performed During Current Reporting Period

- Finalized installation of "Defiant" server. Initial hardware problems corrected.
- Began software inventory system for TOXDET
- Brought "Excalibur" server back on-line. This server is now fully functional.
- Completed phase 1 upgrade of "Trilib" and "Excalibur" servers - phase 2 scheduled for
- next quarter
- Developed and implemented a fully functional WEB server with corresponding WEB page for TOXDET.
- Salvaged parts for and rebuilt Digital multiport repeater thereby saving client several hundred dollars
- Updated Service Packs and Hot Fixes as needed on Network Servers
- Ordered various software and hardware upgrades
- Continued to reconfigure Windows Browser and WINS for WAN as needed
- Continued maintenance of Servers including backing up data files
- Continued support of hardware and software for TOXDET personnel
- Continued to update information Databases
- Created and implemented a new and very successful database to be used by purchasing staff to track travel moneys for upcoming fiscal year and capture old data from past years
- Continued development of ADP SOP manual - this is an ongoing process that will assist NMRI/TD to meet GALP guidelines
- Mr. Horton and Mr. Dibley continue to attend the full MCSE course at Miami-Jacobs College on their own time. Both have completed the Windows NT on the Enterprise course and have completed the corresponding Microsoft certification exam.

Goals/Objectives For Next Reporting Period

- Continue to modify the ADP SOP manual as necessary
- Continue to provide guidelines for installation of network resources for the
- Neurobehavior group at the VA lab as the plan for the network is developed.
- Mr. Horton should attend a comprehensive Network Security course
- Mr. Dibley should attend an in-depth Microsoft Exchange course

JUNG, NARAYANAN

Description Of Work To Be Performed.

During the past quarter, the work carried out by this group was:

Trimethylolpropane (TMPP) Evaluation

- A paper was written on the results of this study and is being reviewed. HPLC analysis of the levels of amino acids in TMPP exposed rat brains was begun. The paper written by Dr. Lindsey was accepted by the journal it was submitted to and returned by the journal review for corrections. These were completed and the paper resubmitted to the journal.

DBNP

- A paper was reviewed by Dr. Carpenter and returned for corrections. These were completed and the paper was sent out for publication approval. The DBNP technical report was also returned for updating. This was completed and the report turned back in. DBNP was synthesized and the melting point and HPLC elution pattern checked to verify purity.

Drug Distribution Study

- A study was proposed by Dr. Nordholm to measure the rate at which five drugs reach the brain using a microdialysis technique. A literature search was conducted to locate HPLC methods for quantitating these drugs from microdialysis samples. The protocol for the experiment is in the process of being written and will be submitted for approval by Dr. Nordholm.

JP-8 Fuel Project

- The standardization of the detection and quantification of JP-8 fuel by Perkin-Elmer GC was begun.

Technical Objectives For The Reporting Period

The objectives for this period were to:

TMPP
- Begin analysis of brain homogenate samples for amino acid content by HPLC
- Complete the corrections to the paper and resubmit it

DBNP
- Synthesize 2 kg of DBNP and verify its purity
- Complete corrections to the technical report and the paper and send both back out for review

Drug distribution study
- Locate the HPLC methods for the detection of the five different drugs and give them to Dr. Nordholm for inclusion in the protocol

JP-8 Fuel Project
- Standardize the GC method by which JP-8 fuel will be quantitated

Summary Of Work Performed During Current Reporting Period

TMPP
- The prepared paper is still in the review process. Ms. Jung began working with Dr. Lindsey on setting up a method of amino acid analysis using a BAS HPLC. The method has been standardized and analysis of samples has started. The quantification of 125 brain homogenate samples for their amino acid content will take approximately 8 - 10 weeks. Dr. Lindsey's paper was corrected and resubmitted to the journal.

DBNP
- This project was completed. We synthesized 2.4 kg of DBNP. The purity of the crystals was verified by melting point and HPLC elution pattern data. The paper has been reviewed in house and returned for corrections. These were completed and it has since been sent out to

the journal for publication. Captain Still also requested that the DBNP technical report be updated. It was written about a year ago. The corrections were completed and reviewed by Dr. Briggs. It was then given to the Captain to be sent out to NMRI.

General

- Dr. Narayanan prepared six research grant proposals. These were sent to prospective funding sponsors.

Drug distribution study

- A literature search was conducted to find hplc analysis methods for the detection of diazepam, ethanol, caffeine, nicotine, and amphetamine. Dr. Nordholm has proposed a study that would use microdialysis techniques to take samples directly from the brain of a rat that has been given one of the drugs listed and then use a hplc method to quantitative the drug administered.

JP-8 Fuel Project

- The Perkin-Elmer GC was brought back into operation. It had been sitting without use for a year. The method by which JP-8 fuel can be measured is in the process of being standardized. Once this has been completed, rats will be exposed to different levels of JP-8 fuel and the level in the blood will be quantitated. The clearance of the compound from the system will also be measured.

Publications, Abstracts, etc.

- "Absorption, Distribution, Metabolism, and Excretion of 2,6-Di-Tertiary-Butyl-4-Nitrophenol in Fischer-344 Rats" TK Narayanan, A. E. Jung, S. L. Prues, R. L. Carpenter and K. R. Still
- "Tissue Distribution, Metabolism, and Clearance of Trimethylolpropanephosphate (TMPP) in Fischer-344 Rats: Tanjore K. Narayanan, Anne E. Jung, Glenn D. Ritchie, John F. Wyman, and John Rossi III.
- "Acute Effects of a Bicyclophosphate Neuroconvulsant on Monoamine Neurotransmitter and metabolite Levels in the Rat Brain" James W. Lindsey, Anne E. Jung, Tanjore K. Narayanan, Glenn D. Ritchie.
- "Characterization of the Metabolism, Distribution and Toxicity of 2,6-Di-Tertiary-Butyl-4-Nitrophenol for the Purposes of Health Hazard Assessment" R.L. Carpenter, T.K. Narayanan, A. E. Jung, S. Prues, and K.R. Still - The DBNP Technical Report

- Research Proposal: "Signal Transduction Mechanisms in Cardiac Toxicity by halogenated hydrocarbons. 3 years- $409K.

Pre-proposals:

- Monoamines, Glucocorticoids and corticotropin releasing factors in prolonged stress.
- Modulation of neuro-endocrine response due to stress by cytokines.
- Phenotypic and neurotypic markers for neurotoxicity by environmental factors.
- Oxidative stress-mediated calcium deregulation as a common mechanism in neuronal cell death.

Goals/Objectives For Next Reporting Period

- Increase the productivity in the lab
- Continue the TMPP binding studies on the benzodiazapine receptor using ^{35}S and ^{36}Cl once a license for these radio labels has been approved
- Continue work on the brain amino acid content analysis
- Begin a study on the enzymes glutamic acid decarboxylase and GABA transaminase, serine -trans-hydroxymethylase, aspartate aminotransferase, and glutamine oxidase
- Begin a study based upon the four endocrine preproposals written earlier
- Begin work on the drug distribution study once it has been approved
- Begin exposing rats and running samples of the JP-8 project.

KIMMEL, REBOULET, WHITEHEAD

Description Of Work To Be Performed

- This group is responsible for the design, construction and operation of systems to conduct inhalation toxicity studies. We also are perform a variety of assays of pulmonary toxicity. Our present research focus is the development and exploitation of small animal models of Acute Lung Injury (ALI) and it's more severe form Acute Respiratory Distress Syndrome (ARDS) as induced by inhalation of combustion atmospheres and surrogate combustion atmospheres. We have developed inhalation exposure systems ranging from highly instrumented, single animal, nose-only exposure chambers suitable for inhalation dosimetry

studies to a large (690 L) whole-body inhalation exposure chamber. We also have and continue to develop methods to measure small animal pulmonary function and perform histopathological analysis of pulmonary tissue samples.

Technical Objectives For The Reporting Period

- Develop methods to analyze ventilation and pulmonary mechanics in six animals simultaneously and in real time during an exposure.
- Develop an exposure system to expose single animals (nose-only) with a non-rebreathing valve in line
- Develop and perform battery of small animal pulmonary function tests using a pressure type plethysmograph,
- Develop protocol for experiments to verify a mathematical model of hyperventilation induced by CO_2.
- Develop protocol for experiments to verify mathematical model of HbCO production by inhalation of CO.

Summary Of Work Performed During Current Reporting Period

- Installed and verified function of a nose only exposure chamber suitable for exposure of 12 animals (6 of which are instrumented for plethysmography) to combustion atmospheres. Measurement capabilities include 16 different flow derived parameters plus standard f, Vt, Ve, Cdyn, RL, elastance, conductance and specific values for each of these.
- Developed and constructed an exposure system for CO2 and CO exposures with real time concentration monitoring and control which will service 3 different exposure chambers, depending upon need.
- Developed methods to determine gas exchange capacity in small animals and determination of ventilation perfusion ratios.
- Designed and developed a plethysmography which will serve as both a plethysmography and exposure holder for nose-only exposure. Our complete redesign of standard methodology permits measurement of intrapleural pressure for RL, and conductance determinations in real time (during exposure) without the need for surgical implantation of a chest wall cannula. We conducted a series of experiments on twenty animals to confirm that the new methodology does not significantly effect other parameters that need to be measured. One

manufacturer of commercially available small animal pulmonary function testing equipment (Buxco Electronics) has expressed an interest in purchasing patent and manufacture rights for this innovation. Dr. Kimmel have requested information and (with no response as of yet) for patent process.

- Developed capabilities to perform barometric plethysmography on awake, free roaming animals while exposing them to gaseous and aerosol atmospheres. Conducted a series of experiments with 25 animals to validate function of this system and develop a data base suitable for use in hypersensitization experiments.
- Developed data base for microcapnometeric determination gas exchange for determination of basal metabolic rate and cardiac output in rats. Collected baseline data on 15 animals.

Publications, Abstracts, etc.

- **Kimmel EC, Yerkes KL and Carpenter RL.** Performance, fluid mechanics, and design of a small animal, whole-body inhalation exposure chamber. *Inhal.Toxicol.* 9(3):287-315. 1997
- **Smith EA, Kimmel EC, English JH, Bowen LE, Reboulet JE, Still KR and Carpenter RL.** The assessment of toxicity after exposure to a pyrotechnically-generated aerosol. *J. Appl. Toxicol.* 17(2):95-103, 1997.
- **Smith EA, Kimmel EC, Bowen LE, Reboulet JE and Carpenter RL.** Preliminary assessment of a pyrotechnically generated aerosol fire suppressant. *Inhal. Tox.* 9:449-463. 1997

Publications in review or in press

- **Kimmel EC, Smith EA, Carpenter RL, Reboulet JE, and Black BH.** Comparison of the potential risk for inhalation toxicity between laboratory and field generated atmospheres of a dry powder fire suppressant
  (Submitted - *Inh. Tox - accepted -in revision*).
- **Kimmel EC, Smith EA, Reboulet JE and Carpenter RL.** Application of physiological interactions with dry powder fire suppressant atmospheres for health risk assessment: Implications for pulmonary deposition and toxicity. (Submitted - *J Appl Physiol.)*
- **Kimmel EC, Reboulet JE and Carpenter RL.** Inhalation exposure chamber leak rate determination with thermal correction. (*Am Ind. Hygiene Assoc. J.* - in press).
- **Reboulet, JE, Kimmel EC and Carpenter RL.** A basic computer program for rapid standard bag calculations. (*Tox. Methods* - submitted).

Publications in draft

- **Kimmel, Reboulet, Narayanan, Carpenter.** The Effects of co-exposure with aerosol particles on acrolein inhalation toxicity. Toxicol Appl Pharmacol.
- **Kimmel.** A head out plethysmography for measurement of intrapleural pressure and determination of pulmonary mechanics in small animals during inhalation exposure without surgical intervention. J Appl Physiol.

Technical Reports in press

- **Kimmel EC, Smith EA, Reboulet JE, Still KR, and Carpenter RL**. 1997. The physicochemical properties of safe fire suppressant atmospheres in toxicity vs. fire extinguishment tests: Implications for aerosol deposition and toxicity. 43pp.
- **Kimmel EC, and Still KR**. 1997. The acute respiratory distress syndrome (ARDS) and militarily relevant inhalation injury: A brief review., 88pp.

Abstracts and presentations given

- Pulmonary toxicity of co-exposure to acrolein and aerosol particles in F-344 rats - II. 36th Annual meeting of the Society of Toxicology. Cincinnati, OH. March 1997.
- Edemagenesis in f-344 rats exposed to SAFE (Formulation A)Atmospheres. 36th Annual meeting of The Society of Toxicology. Cincinnati, OH. March 1997.
- The acute respiratory distress syndrome (ARDS): Inhalation Injury. 38th Navy Occupational Health and Preventative Medicine Workshop. Virginia Beach, VA - February 1997.
- Pulmonary Toxicity of co-exposure to acrolein and aerosol particles in F-344 rats. 31st Conference on Toxicology, Conference on Issues and Applications in Toxicology and Risk Assessment. Wright-Patterson AFB, OH - April, 1997.
- Concepts in the inhalation toxicology of fire suppressants: Pneumotoxicity. Halon Options Technical Working Conference - Albuquerque, NM - May 1997.

Invited presentations and working group

- Invited by National Institutes of Standards and Technology to make recommendations assessment of toxicity required for the new generation of Halon replacement candidates.

Number of abstracts submitted for future presentations

- SOT - 2
- Conference on Toxicology - WPAFB - 3 - Inhalation toxicology co-chair.
- JANNAF -1
- Invited speaker at HOTWC (Halon Options Technical Working Conference)

Goals/Objectives For Next Reporting Period

- Finish development of battery of pulmonary function tests. Tests to be added to the regimen are forced maneuvers (FEV, FVC,MEFV, flow- volume loop), N_2 washout, non-invasive continuous diffusing capacity. (multiple breath).
- Conduct an experiment needed to verify mathematical model (logistic model using - physiologic constants) of CO_2 induced hyperventilation in animals. This model will be used for risk assessment and prediction of increased dosimetry to other toxins in complex atmospheres which contain CO_2.
- Conduct an experiment needed to verify mathematical model predictive of HbCO formation in animals exposed to CO. This model is needed for extrapolation of data from animal studies to human risk assessments and predicting toxicity of combustion atmospheres.
- Complete first drafts of:
- manuscript on the development of a new plethysmographic technique - to J of Applied Physiology
- technical reports - on measurement of small pulmonary function in NMRI/TD system - will do a series of reports describing fundamentally different techniques - at present 2 reports have been started addressing barometric methods and a second flow plethysmographic methods for determination of ventilation and dynamic mechanics of breathing.
- manuscript describing an small scale in expensive solid material combustion furnace for laboratory combustion toxicology studies that was developed by our group - submission to J Fire Sciences.

MCINTURF

Description Of Work To Be Performed

- Mr. McInturf serves as the research design and analysis assistant for the Neurobehavioral Group at the NMRI/TD, and as a primary research technician of data collection for all currently funded neurobehavioral research.
- Mr. McInturf's purpose is to assist in the design, data collection, and analysis for most of the neurobehavioral studies. He is also involved with the fabrication, setup, and operation of hardware/software and other instrumentation for data collection.

Technical Objectives For The Reporting Period

- To collect and analyze data from rabbit eyeblink conditioning studies to evaluate the effects that neurotoxins (TMPP & PTZ) have on conditioned response and/or learning and memory. Also look at the possible counteractive or preventive capacities that well known human anticonvulsant agents may have on neurotoxins.
- Aid in the design, collection of data, and analysis for a study of repeated exposure to TMPP on acoustic startle, prepulse inhibition and acoustic startle habituation in rats.
- Complete design of Morris-like water maze to be used in up-coming neurobehavioral study involving neurotoxins and possible counteracting drugs.
- Focus on program languages used with current hardware (particularly MED-PC notation) in order to write programs and macros for operant and other studies.
- Continue to offer research design and data analysis assistants via the use of statistical software packages.
- To continue relocation of the NMRI/TD Neurobehavioral Laboratory to the Research Facility of the Veterans' Administration Hospital, Dayton, OH.

Summary Of Work Performed During Current Reporting Period

- Design, data collection, and analysis for acoustic startle pre-pulse inhibition/habituation and TMPP study.
- Data collection and analysis for rabbit eyeblink study to determine the effects of convulsants and anticonvulsant agents on conditioned response and learning and memory.

- Performed stereotaxic surgery for placement of bipolar electrodes in the nucleus accumbens or ventral tegmental area for evaluation of TMPP-induced effects on intracranial self-stimulation.
- Designed and constructed a Morris-like water maze pool with frame to present visual cues.
- Setup and operation of all hardware and instruments for water maze, acoustic startle, and conditioned eyeblink response.
- Completed literature surveys on eyeblink, acoustic startle, and water maze as relevant to
- NMRI/TD research.
- Continued familiarization of programming languages and a variety of software applications that run on many of the data collecting instruments.

Presentations, Abstracts, etc.

- Nordholm, A., Ritchie, G., MacMahon, K., Hulme, M.B., **McInturf, S.**, and Rossi III, J. Acute and long-term consequences from repeated exposure of rats to JP-4 jet fuel and/or stress. *Abstract*, Society of Toxicology, Annual Meeting, Seattle, WA, 1998.

Goals/Objectives For Next Reporting Period

- To continue with and complete all current studies involving the effects of convulsants and anticonvulsants in rats and rabbits and perform a critical analysis.
- Implantation of canula in rabbits to further study effects of neurotoxins and anticonvulsants when administered directly in brain.
- To compete all aspects of water maze construction and assist in experimental design of
- relevant NMRI/TD study.
- To assist in slide preparation and analysis for studies on hippocampal tissue slice and TMPP exposure.

HULME

Description Of Work To Be Performed

- The neurobehavioral research technician is responsible for collection and analysis of data from major research studies. During the quarter the technician is responsible for assisting with publications.

Technical Objectives For The Reporting Period

The major technical objectives for this quarter are as follows:

- Begin data collection, analysis, and publication of a major study evaluating the relative capacities of well-known anticonvulsant agents combined with a GABA B antagonist to prevent or counteract neurotoxicity induced by exposure to low or high doses of trimethylopropane phosphate.
- Assist in completion of study of repeated exposure to TMPP on acoustic startle, prepulse inhibition, and acoustic startle habituation.
- Begin the study of effects of 9 neurotoxins in rats as measured by Navy Roto-Wheel performance.
- Present Society of Neuroscience poster.
- Assist in the operant conditioning of rats and pigeons.
- Complete study of TMPP-induced effects on learning and performance of the conditioned eyeblink response in rabbits.
- Complete study of rats with bipolar electrodes in nucleus accumbens or ventral tegmental area for evaluation of TMPP-induced effects on intracranial self-stimulation.
- Begin set up of new database for Reference Manager.
- Do literature searches for upcoming protocols.

Summary Of Work Performed During Current Reporting Period

The major accomplishments for this quarter are as follows:

- Completed data collection, and analysis of the study evaluating the relative capacities of well-known human anticonvulsant agents combined with a GABA B antagonist to prevent or counteract absence-like seizures induced by exposure to doses of trimethylopropane phosphate.
- Completed data collection, and analysis of the study evaluating the effects of ethanol on Navy Roto-Wheel performance in rats.
- Completed study of rats with bi-polar electrodes in nuclear accumbens or ventral tegmental for evaluation of TMPP- induced effects on intracranial self stimulation.
- Completed literature searches for all upcoming protocols.
- Completed study of TMPP-induced effects on learning and performance of the conditioned eyeblink response in rabbits.

- Presented Society of Neuroscience Poster.
- Completed standard operating procedures for EEG and Roto-Wheel.

Presentations, Abstracts, etc.

- Ritchie, G.D., Hulme, M.B., Nordholm, A.F. and Rossi III, J. Effects of GABA B antagonist CGP-35348 and human anti-epileptic drugs on spontaneous and chemically induced absence-like SDWs in Fiser-344 rats.
- Nordholm, A., Ritchie, G., MacMahon, K., Hulme, M.B., McInturf, S., and Rossi III, J. Acute and long-term consequences from repeated exposure of rats to JP-4 jet fuel and/or stress. *Abstract*, Society of Toxicology, Annual Meeting, Seattle, WA, 1998.
- Ritchie, G.D., Rossi, J., Hulme, M.B., Ademujohn, C.Y. and Cassel, J. Effects of GABA B antagonist CGP-35348 and human anti-epileptic drugs on spontaneous and chemically induced absence-like SDW's in Fisher-344 rats. *Poster Presentation*, Society for Neuroscience Annual Meeting, New Orleans, LA, Oct 1997.

Goals/Objectives For Next Reporting Period

The major objectives for next quarter are as follows:

- Assist in completing publication of the study evaluating the relative capacities of well-known human anticonvulsant agents combined with a GABA B antagonist to prevent or counteract absence-like seizures induced by exposure to doses of trimethylopropane phosphate.
- Complete data collection, analysis of the study evaluating the effects of diazepam on Navy Roto-Wheel performance in rats.
- Continue literature searches as needed to prepare for upcoming studies.
- Complete set up of new database for Reference Manager.
- Assist in study of TMPP-induced effects on learning and performance of the conditioned eyeblink response in rabbits.
- Assist in the set up of juvenile play analysis equipment.
- Assist in operant conditioning of rats and pigeons.
- Assist in completion of Porsalt Swim Test of Anxiety and Depression.

SMITH, PRUES

Description Of Work To Be Performed

Assist in the research performed at the Navy Medical Research Institute/ Toxicology Detachment (NMRI/TD) which entails the following tasks:

- Conduct/ design /oversee studies addressing Navy related research issues.
- Provide necessary paperwork for the accounting of project funding
- Maintain GLP compliant data books on those studies with which there is personal involvement.
- Submit articles/revisions for publication of project findings to peer-reviewed journals.
- Submission of timely progress reports

Technical Objectives For The Reporting Period

Technical support for the following NMRI/TD projects is to be provided for:

Spectrex Fire Extinguishant (SFE)

- The objective of this research is to evaluate the potential health effects of exposure to the by-products of pyrolyzed SFE. SFE is a fire suppressant and a potential replacement for Halon 1301.

Cardiac Sensitization

- The objective of this research is to develop a model for the determination of cardiac sensitization. These initial studies will set the basic background needed for future studies.

Trimethylopropane phosphate (TMPP)

- The objective of this research is to determine the mechanism of action of TMPP. TMPP is a by-product from the breakdown of synthetic lubricants that produces a neurotoxic response.
- Contract Representative on the Safety Policy Committee (Sue Prues)
- The objective of this duty is to act as liaison between the Navy and Geo-Centers personnel in addressing the concerns of workplace related safety issues.
- Technical support for the Air Force Aircraft Composite Material (ACM) project is to be provided.

Summary Of Work Performed During Current Reporting Period

SFE Project -- Homeostasis and Edema

- Concluded baseline blood gas studies involving homeostatic effects following a serial blood collection/transfusion.
- Graphic and statistical analysis/presentation of data

Cardiac Sensitization

- Continue probing the mechanical and electrophysiological events leading to ventricular fibrillation using the swine model.
- Began dog studies involving chemical blockage of the vagus nerve
- Wrote pre-proposal to continue cardiac sensitization research (Evaluating Cardiac Sensitizing Agents with Respect to Vagal Influence on the Heart)

TMPP Project -- Glial Fibrillary Acidic Protein (GFAP)

- Wrote pre-proposal for developing GFAP microassay (The Development of Neuro Proteins Microassays)

Intercranial Selfstimulation (ICSS)

- Performed EEG electrode implantation surgeries on test rats
- Established baselines and began dose curve studies.

General

- Provide assistance/training for projects requiring drug preparation, surgical implantation of devices via stereotaxic methodology, etc. as needed

ACM Project

- Collected Branchoalveolar lavage (BAL) and tissue samples from animals involved in the Air Force Inhalation study.

Publications, Abstracts, etc.

- Abstracts (submitted to The Society of Toxicology for the upcoming meeting in the Spring of 1998)

- The Evaluation of Blood Transfusions During Serial Blood Sampling. S L Prues, E A Smith, and A F Walsh. Naval Medical
- Predicting Cardiac Sensitization Using Logistic Regression on Cardiovascular Parameters. E A Smith, T Nakayama, R Hamlin, J Powers, E Herderick and K R Still.
- Hard copies of this years' publications will be provided in the packet.
- Preliminary Assessment of a Pyrotechnically Generated Aerosol Fire Suppressant. Eldon A. Smith, Edgar C. Kimmel, Larry E. Bowen, James E. Reboulet, and Robert L. Carpenter, Inhalation Toxicology, (1997) 9:449-463.
- Evaluation of the Respiratory Tract after Acute Exposure to a Pyrotechnically Generated Aerosol Fire Suppressant. Eldon A. Smith, Edgar C. Kimmel, Jeffery H. English, Larry E. Bowen, James E. Reboulet, and Robert L. Carpenter, Journal of Applied Toxicology, (1997) 17(2):95-103.
- Comparison of Toxicity after Exposure to Two Formulations of SFE Formulation A. Eldon A. Smith, Edgar C. Kimmel, James E. Reboulet, Susan L. Prues, and Robert L. Carpenter, Fundamental and Applied Toxicology (in review).

Goals/Objectives For Next Reporting Period

- Development of a GFAP immunoassay (to investigate subtle changes in the central nervous system) for the TMPP project
- Continue the dose curve response for the ICSS study
- Continue providing general assistance and training where necessary for the TMPP project
- Analyse the BAL and tissue samples colleced for the Air Force ACM project
- Continue representation of Geo-Centers concerns regarding health and safety.
- Conduct/support pulmonary physiology studies particularly the upcoming study involving cross comparison of traditionally collected blood samples to serially collected blood samples for the purpose of blood gas analysis.
- Continue writing new protocols in the area of pulmonary toxicology.

V. NMRI, Natick, MA

A. HUMAN PERFORMANCE AND U.S. NAVY CLOTHING DEVELOPMENT

Description Of Work To Be Performed

Program I: Flame Protective Clothing Research **(Pawar)**

- The primary research goal for the current reporting period was to complete calibration routines for the automation of Thermal Protection Performance (TPP) equipment, demonstrate the Burn Injury Sensor Calibration System (BISC) for its usefulness in the selection of a sensor to suit a given fire hazard and validate the Wissler math model for additional data on rough and calm seas. However, due to the high priority given to completion of math modeling project, the TPP sensor calibration and development of the BISC system could not proceed as planned. Therefore, major part of this quarter was spent on Math Modeling Project which is completed to a stage of writing final report.

Program II: U.S. Navy Certification Program for Commercial Environmental/Occupational (CEO) Protective Clothing/Equipment **(Macek)**

- GEO-CENTERS, INC. will establish a program to be used by NCTRF to certify commercial off-the-shelf protective clothing/equipment as meeting or exceeding Navy functional performance requirements. This program will make possible the direct purchase of certified commercial protective clothing/equipment for shipboard use by Navy personnel.

Program III: Database Search **(Macek, Collins)**

- Conduct an extensive search of databases to determine commercial, DoD and non-DoD government organizations with which the U.S. Navy Clothing & Textile Facility (NCTRF) may enter into cooperative R&D agreements for the research, development, and testing of dress and protective clothing systems.
- Determine cooperative opportunities for dual-use technology, technology transition, and technology exploitation.
- Prepare a technical briefing to highlight the technical expertise and unique facilities and equipment available at NCTRF. This briefing could be used by agencies seeking cooperative research, development, and acquisition agreements.

- Prepare documentation to convey the technical expertise and unique facilities and equipment available at NCTRF. This documentation could be used by NCTRF employees to serve as a marketing tool and as a handout after the technical briefing is presented.

Program IV: Great Lakes Prototype Footwear Test **(Buller, Collins)**

- Provide technical support in the development of the Enhanced Chukka Shoe surveys for recruits, leaders, shipboard personnel, and Naval Academy personnel.
- Provide technical support for experimental design of study.
- Provide software support in the production of an on-line data entry program and database management.
- Provide data collection support at the Recruit Training Center (RTC).
- Analyze data by test group and write final report of findings of the study.

Program V: Technical Reports **(Macek, Schneider)**

- Analyze and organize information provided on projects conducted in the Navy Clothing and Textile Research Facility (NCTRF).
- Develop technical reports and articles for publication in peer-reviewed journals.

Program VI: Utility Uniform Study **(Buller, Meyers, Collins)**

Commercial-Off-the-Shelf Utility Uniform Study

- Design questionnaire to assess fit, performance, durability and preference for two commercial off- the-shelf utility uniforms. The two styles are: 1) Redcap, and 2) Levi 505.
- Produce issue data sheets and explanatory package for subjects.
- Reproduce questionnaires and issue packages.
- Analyze data.
- Write final report.

Main Utility Uniform Study

- Adapt questionnaire, data sheets, and explanatory package from COTS study for three uniform configurations: 1) 14 oz. Denim with 4 oz. Chambray Shirt, 2) 11 oz. Denim with 4 oz. Chambray Shirt, and 3) "Dickie" Style.
- Reproduce questionnaires and issue packages for all test participants.
- Provide support of two issuers to 16 test sites on the East and West Coasts, with approximately 75 test participants at each site.
- Provide support of two Human Factors Engineers to visit each test site twice during the duration of the study to issue and collect surveys and to collect subject comments. Visits will occur three and six months after issue of utility uniforms.
- Enter, clean, verify, and tabulate collected data.
- Analyze data based upon experimental design and study hypothesis, using standard univariate and multivariate statistical techniques.
- Write report detailing whole study providing a clear explanation of the analytical techniques adopted and the conclusions reached from analysis of the data.

Program VII: Oxford Shoe Study **(Buller, Stern-Wolfson)**

- Design questionnaire to assess fit, performance, durability and preference for three Oxford shoe sole configurations.
- Design issue data sheets.
- Enter, clean, verify, and tabulate collected data.
- Analyze data based upon experimental design and study hypothesis, using standard univariate and multivariate statistical techniques.
- Write report detailing whole study providing a clear explanation of the analytical techniques adopted and the conclusions reached from analysis of the data.

Technical Objectives For The Reporting Period

Program I: Flame Protective Clothing Research

- None.

Program II: U.S. Navy Certification Program for Commercial Environmental/Occupational (CEO) Protective Clothing/Equipment

- Complete editorial changes to the certification program report and submit to NCTRF for review and comment.

Program III: Database Search

- Complete final revisions to prototype brochure, folder cover, and information sheets.

Program IV: Great Lakes Prototype Footwear Test

- None.

Program V: Technical Reports

- To conduct work on two reports for the Navy Clothing and Textile Research Facility.

Program VI: Utility Uniform Study

COTS Study

- Analyze all data.
- Produce integrated final report for both COTS study and main study.

Main Utility Uniform Study

- Report analysis of data in full integrated technical report.

Program VII: Oxford Shoe Study

- Enter, verify, and clean all phase two and most of phase three data.

Summary Of Work Performed During Current Reporting Period

Program I: Flame Protective Clothing Research

- None.

Program II: U.S. Navy Certification Program for Commercial Environmental/Occupational (CEO) Protective Clothing/Equipment

- None.

Program III: Database Search

- Revised draft brochure, folder cover, and information sheets in accordance with suggested changes obtained from NCTRF and produced a final product.
- Delivered electronic files for use to produce high quality brochures, folder covers, and information sheets.

Program IV: Great Lakes Protective Footwear Test

- None.

Program V: Technical Reports

- Work was continued on the report which dealt with the development of a laboratory method of rough sea simulation for the immersion testing of protective clothing using a Wave Maker that was installed in the NCTRF environmental tank. A draft of the technical report, titled *Comparison of Field and Laboratory Tests of Body Cooling Rates Using a Wave Maker to Simulate Rough Seas*, has undergone full editorial review.
- Worked continued on the second report entitled, *Pumped Fluid System for Body Heat Transfer.* Some references, to be supplied by the Project Officer are needed to complete the article.

Program VI: Utility Uniform Study

COTS Study

- All data from the COTS study were analyzed using standard statistical techniques.
- Report on findings was integrated into final technical report.

Main Utility Uniform Study

- All data were combined and analyzed using standard statistical techniques.
- Final technical report of study was produced.

Program VII: Oxford Shoe Study

- All phase two data and all phase three data which had been returned were entered, verified, and cleaned.

Goals/Objectives For Next Reporting Period

Program I: Flame Protective Clothing Research

- None.

Program II: U.S. Navy Certification Program for Commercial Environmental/Occupational (CEO) Protective Clothing/Equipment

- Upon receiving comments from NCTRF on the certification program report, GEO-CENTERS, INC. will incorporate the changes into the report.

Program III: Database Search

- None.

Program IV: Great Lakes Protective Footwear Test

- None

Program V: Technical Reports

- Complete work on the two projects described above and submit the draft technical reports to the Project Officers for review.
- Begin work on an article dealing with the NCTRF studies of laboratory rough sea simulation methods for publication in a peer-reviewed technical journal.

Program VI: Utility Uniform Study

COTS Study

- None. Study and final report have been completed.

Main Utility Uniform Study

- None. Study and final report have been completed.

Program VII: Oxford Shoe Study

- Analyze combined data set.
- Provide summary report of combined data.
- Write final technical report.

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Evaluation of the Respiratory Tract after Acute Exposure to a Pyrotechnically Generated Aerosol Fire Suppressant

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Key words: fire suppressant; aerosol; pulmonary edema

Fischer 344 rats (250–300 g) were exposed to the resulting aerosols from the pyrolysis of Spectrex Fire Extinguishant (SFE) Formulation A, a pyrotechnically generated aerosol fire suppressant, at a loading equivalent of 50 or 80 g m^{-3} air for 15 or 60 min. Exposures were conducted in a 700-l whole-body inhalation chamber under static conditions. The chamber atmosphere was analyzed for mass aerosol concentration and size distribution. Clinical observations were taken throughout the exposure. Animals were euthanized at 1 h, 6 h, 24 h, 7 days or 14 days post-exposure and underwent histopathological examination, enzyme analyses and wet/dry lung weight determination. No deaths occurred during the study. Animals exhibited signs of dyspnea, coughing, lack of coordination and lethargy during each exposure. These signs became more pronounced as the load and exposure length increased. No lesions were noted in the trachea, lung, heart or abdominal organs upon gross examination. A reversible pulmonary edema and olfactory necrosis were observed only in those animals exposed to an SFE loading equivalent to 80 g m^{-3} for 60 min. Protein concentrations increased in the bronchoalveolar lavage but no changes in enzyme levels were observed. There was no significant difference between the control groups and the exposure groups for wet/dry lung weight determination. © **1997 John Wiley & Sons, Ltd.** ***J. Appl. Toxicol.*****, Vol. 17(2) 95–103 (1997)**

(No of Figures: 5. No of Tables: 2. No of Refs: 14)

INTRODUCTION

During the suppression of a fire, chlorofluorocarbons (CFCs) and halocarbons (Halons) are unintentionally released into the environment. The release of such fluorocarbons has been shown to indirectly destroy atmospheric ozone.[1] Because of this destructive effect, countries around the world have agreed to slowly phase out the production (1987 Montreal Protocol) and eventual use of CFCs and Halons. With the impending halt of CFC and Halon use, fire extinguishant manufacturers have begun to investigate alternative forms of fire suppression, such as dry-powder, aerosol fire suppressants.

The fire-suppressive capabilities of aerosols are derived from various mechanisms (i.e. vaporization, decrepitation, decomposition and surface mediated phenomena),[2] physical properties (i.e. mass concentration, size distribution and specific surface area (SSA)) and chemical properties (i.e. particle density, morphometry and chemical composition)[3–5] of the aerosol. These dry-powder, aerosol fire suppressants are available in the following delivery systems: powder/gas mixture dispersal systems and pyrotechnically generated aerosol systems. Spectrex Fire Extinguishant (SFE) is an example of a pyrotechnically generated aerosol.

Pyrotechnically generated aerosols are formed when the parent (or starting) material reaches an initiation temperature. In the case of SFE, this temperature is ~500°C. Once the initiation temperature is reached, the parent material undergoes a cascade of chemical reactions which ultimately results in the production of the aerosol. Spectrex Fire Extinguishant Formulation A (SFE-A) is comprised primarily of potassium perchlorate, which upon decomposition produces an aerosol of potassium chloride.[6] The aerosol particles produced during the pyrolyzation (i.e. evaporation/condensation) process are typically of a respirable size. Respirable size refers to a particle that is 1–10 μm in diameter. These particles, when inhaled, are capable of penetrating deep into the tracheobronchial tree. Therefore, particle size is important in determining the toxicity of an aerosol.

The specific surface area and chemical solubility of the particulate material are also important in determining the toxicity of aerosols. As is true of other administration routes, the lungs' response to dissolved material depends highly on the chemical composition of the particulate. Particulates that are a salt or have a high pH may irritate the lung and cause imbalances in hydrostatic forces, thereby altering pulmonary function. During the pyrolyzation process, pyrotechnically gener-

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ated aerosols may also release combustion gases, such as carbon monoxide. Exposure to combustion gases such as carbon monoxide will further alter gas exchange, thereby leading to disorientation and incapacitation at high doses. Therefore, both the physical properties and chemical composition of the aerosol can dictate the overall toxicity of the aerosol. Direct dermal, ocular or nasal contact with the aerosol may result in irritation of that site.

In a previous pilot study, Fisher 344 rats were exposed to the resulting aerosol from pyrolyzed SFE-A and evaluated for 1 h post-exposure.[7] Loadings of 50 and 80 g m^{-3} were used. These values were selected based upon manufacturer's recommendations and fire suppression data. The exposures were conducted in a whole-body inhalation chamber under static conditions for 15 or 60 min. No deaths occurred during the exposure and observation period. During the exposure to SFE, animals exhibited signs of dyspnea, lack of coordination and lethargy. These signs became more pronounced as the load and exposure length increased. Tissue and blood samples were collected 1 h post-exposure. No lesions were noted in the trachea, lung, heart and abdominal organs during gross examination. No abnormalities were noted in the histopathology examination of the turbinates. An increase in carboxyhemoglobin, methemoglobin and serum glucose was observed, as well as a decrease in oxyhemoglobin, deoxyhemoglobin and pH. All other serum chemistry parameters were within their respective biological ranges. Wet/dry lung weight ratio showed no difference between control and exposure groups. Upon pyrolyzation, SFE-A produced aerosol particles 2–3 μm in mass mean aerodynamic diameter (MMAD) at concentrations of 6.6–8.5 g m^{-3}. Therefore, the objective of this study is to examine the possible nasal irritation and pulmonary edema formation from exposure to the by-products of SFE-A between 1 h and 14 days post-exposure.

MATERIALS AND METHODS

Chemicals Spectrix Fire Extinguishant Formulation A was supplied by Spectronix Ltd. (Tel Aviv, Israel)

Inhalation chamber configuration and operation

The exposure system consisted of a modified Hinners-type 700-l inhalation chamber with a supply/exhaust system, a specially designed aerosol generator and an exhaust scrubber.[8] The generator was connected to the inlet side of the system by a 3″ aluminum duct. The system was operated in dynamic mode during the pyrolyzation of SFE until the generation of aerosol had ceased and the chamber was filled and at equilibrium. Control valves were installed in the exhaust, inlet and generator flow lines to transform the chamber from a dynamic to a static system. Exposures were conducted under static conditions. The aerosol generator consisted of two flanged 4″ sections of schedule 80 stainless-steel pipe bolted together. A 1/8″ thick sintered stainless-steel plate was located between the two sections of pipe. Air entered the generator through the lower chamber and passed through the sintered plate into the ignition chamber. The ignitor consisted of a 6-cm piece of 26-gauge nichrome wire attached to insulated copper electrodes and placed through the wall of the upper plenum. The nichrome wire was coiled to fit in the bottom of a ceramic combustion boat placed on the sintered plate. A current of 6 A 918 V) was passed through the nichrome to produce a temperature of 550–600°C. A thermocouple was placed 5 cm above each SFE pellet to monitor the ignition/combustion temperature. Aerosol samples were collected from sampling ports located in the rear of the chamber. Samples were analyzed for concentration, particle size (MMAD) and particle size distribution (σg). The system was exhausted through a scrubber at the conclusion of each exposure.

Aerosol nomenclature

With respect to pyrotechnically generated aerosols, the fire extinguishing community discusses fire protection design in terms of the amount of parent material needed (discharged) per unit volume space. These units, unfortunately, coincide with those commonly used to describe mass aerosol concentrations in an inhalation chamber. To reduce the ambiguity, we have adopted the following nomenclature. The amount of parent (starting) material per cubic meter of space protected is referred to as the SFE load. It is also used to distinguish the various exposure groups. The resulting aerosol measurements, made during the inhalation exposure, are referred to as the mass aerosol concentration and are given the units commonly associated with such measurements. The results of our work show that the two values do not always parallel each other.

Aerosol concentration

The exposure aerosol mass concentration was determined using filter samples. Samples were collected on a 47-mm Gelman 61631 A/E glass-fiber filter that was stored in a dessicator prior to use. Filters were weighed on a Cahn C-31 microbalance (Fisher, Cincinnati, OH) and placed in a brass filter-holder (IN-TOX Products, Albuquerque, NM). Samples were collected at 1, 5 and 15 min for 15-min exposures, and at 1, 5, 15, 30, 45 and 60 min for 60 min exposures. The flow rate through a filter was 5 l min^{-1}, with a sampling time of 15 s.

Particle size distribution and analysis

Mass-weighted aerodynamic particle size distribution was determined using a cascade impactor (IN-TOX Products, Albuquerque, NM). The impactor designs were based on Marple's critera.[9] Aerosol particles were collected on 37-mm stainless-steel substrates coated with apiezon grease to minimize particle bounce. A 47-mm Gelman 61631 A/E glass-fiber filter was used as a final filter. Substrates and filter were weighed on the Cahn C-31 microbalance. Samples were collected at the beginning and end of each exposure. The flow rate through the impactors was at 20 l min^{-1}, with a sampling time of 4–15 s, depending upon the time the sample was collected. Particle size was reported as MMAD and particle size distribution as σg. Real-time

aerosol size distribution was also determined with a TSI model 3300 Aerodynamic Particle Size analyzer (APS; TSI Corp., St. Paul, MN). The APS measured particle number as a function of aerodynamic diameter and computed the MMAD on the basis of equivalent sphere and material density as input to the instrument. Two TSI model 3302 diluters, each with a 100:1 dilution probe, followed the APS, providing a final dilution of 10 000:1. The real-time aerosol analysis was performed on a Zenith Data System 286 PC with TSI model 390041 APS Advanced Software, version B, using a density value of 2 g cc^{-1}. Samples were collected for 10 s at 1 and 15 min for a 15-min exposure, and at 1, 15, 30, 45, and 60 min for a 60-min exposure.

Animals

Twenty male Fisher CDF (F-344)/CrlBR rats were obtained from Charles River Breeding Labs (Wilmington, MA). The rats weighed 200–250 g. Upon arrival the rats were tail tattooed and quarantined for 2 weeks. Animals were housed in a suspended shoebox-type cage. They were provided Formula Lab Chow 5008 (Purina Milles Inc., St. Louis, MO) and reverse-osmosis filtered water *ad libitum*.

Experimental design

Animals were randomized first into five exposure groups and then five post-exposure time points. Each exposure group/post-exposure time point consisted of 14 animals; five animals for histopathological examination, five for enzyme analysis and four for wet/dry lung determination.

Clinical observations

Clinical signs were recorded during the exposure at 1, 5, 15, 30, 45 and 60 min.

Histopathology

Animals were euthanized by an intraperitoneal injection of a Ketamine™ and Xylazine™ mixture: 70 mg kg^{-1} Ketamine™ (Vetalor; Parke-Davis, Morris Plains, NJ) and 6 mg kg^{-1} Xylazine™ (Rompun; Mobay Corporation, Shawnee, KS). The abdominal and thoracic cavities were incised and a gross examination performed on the trachea, lung, heart and abdominal organs. The trachea and lungs were removed from the thoracic cavity and trimmed. To examine nasal turbinates, the head was removed and cut transversely at the level of the incisive papilla and second palatal ridge using a Buehler Isomet low-speed saw with diamond wafering blade (Evanston, IL). All tissue sections were placed in 10% neutral-buffered formalin and decalcified for 3 days in 10% ethylenediaminetetraacetic acid (EDTA; Sigma, St. Louis, MO). The tissues were processed for histological examination (light microscopy). Each section was embedded in paraffin, sectioned at 3–4 μm and stained with hematoxylin and eosin.

Table 1. Experimental Design

Group	SFE load (g m^{-3})	Length of exposure (min)	Post-exposure observation time points 1 h	6 h	24 h	7 D	14 D
1	Control	60	5/5/4[a]	5/5/4	5/5/4	5/5/4	5/5/4
2	50[b]	15	5/5/4	5/5/4	5/5/4	5/5/4	5/5/4
3	50	60	5/5/4	5/5/4	5/5/4	5/5/4	5/5/4
4	80[c]	15	5/5/4	5/5/4	5/5/4	5/5/4	5/5/4
5	80	60	5/5/4	5/5/4	5/5/4	5/5/4	5/5/4

[a]Histopathology (five animals)/enzyme analysis (five animals)/wet and dry lung weight determination (four animals).
[b]Lowest concentration effective for fire suppression.
[c]Concentration recommended by the manufacturer for fire suppression.

Enzyme analysis

Bronchoalveolar lavage (BAL) was collected and analyzed for protein and selected enzymes. Rats were euthanized with an injection of a Ketamine™/ Xylazine™ mixture. The thoracic cavity was incised and the trachea was exposed and cannulated. The lungs were infused three times with 5 ml of Ca–Mg-free phosphate-buffered saline (PBS) (pH 7.2). The BAL from each rat was pooled and centrifuged at 300 *g* for 10 min. The cell pellet was suspended in 5 ml of RPMI 1640 (Gibco, Grand Island, NY) containing 25 mM HEPES buffer. The supernatant was analyzed for total protein content, acid phosphatase, alkaline phosphatase, lactate dehydrogenase, β-glucuronidase, alanine aminotransferase and 5′-nucleotidase.

Wet/dry lung weight determination

Wet/dry lung ratio was measured by the method described by Staub (1974).[10]

Statistical analysis

A single factor analysis of variance with the Bonferroni multiple comparison test was performed on all enzyme and wet/dry lung weight ratio parameters ($P<0.05$). The equality of variance was tested using Levene's test.

RESULTS

Inhalation chamber operation

A pressure pulse was noted 10–15 s after ignition and lasted for 5–10 s. Temperature within the generator ranged from 590 to 815°C for an SFE load of 50 g m^{-3} and from 895 to 1100°C for an SFE load of 80 g m^{-3}. However, the chamber temperature remained at 22–26°C.

Aerosol characterization

The mass aerosol concentrations for SFE loads of 50 and 80 g m^{-3} are shown in Fig. 1. The aerosol concentrations exhibited an exponential decay. The half-life of the aerosol concentration decay was 18.3 min for an SFE load of 50 g m^{-3} and 14.5 min

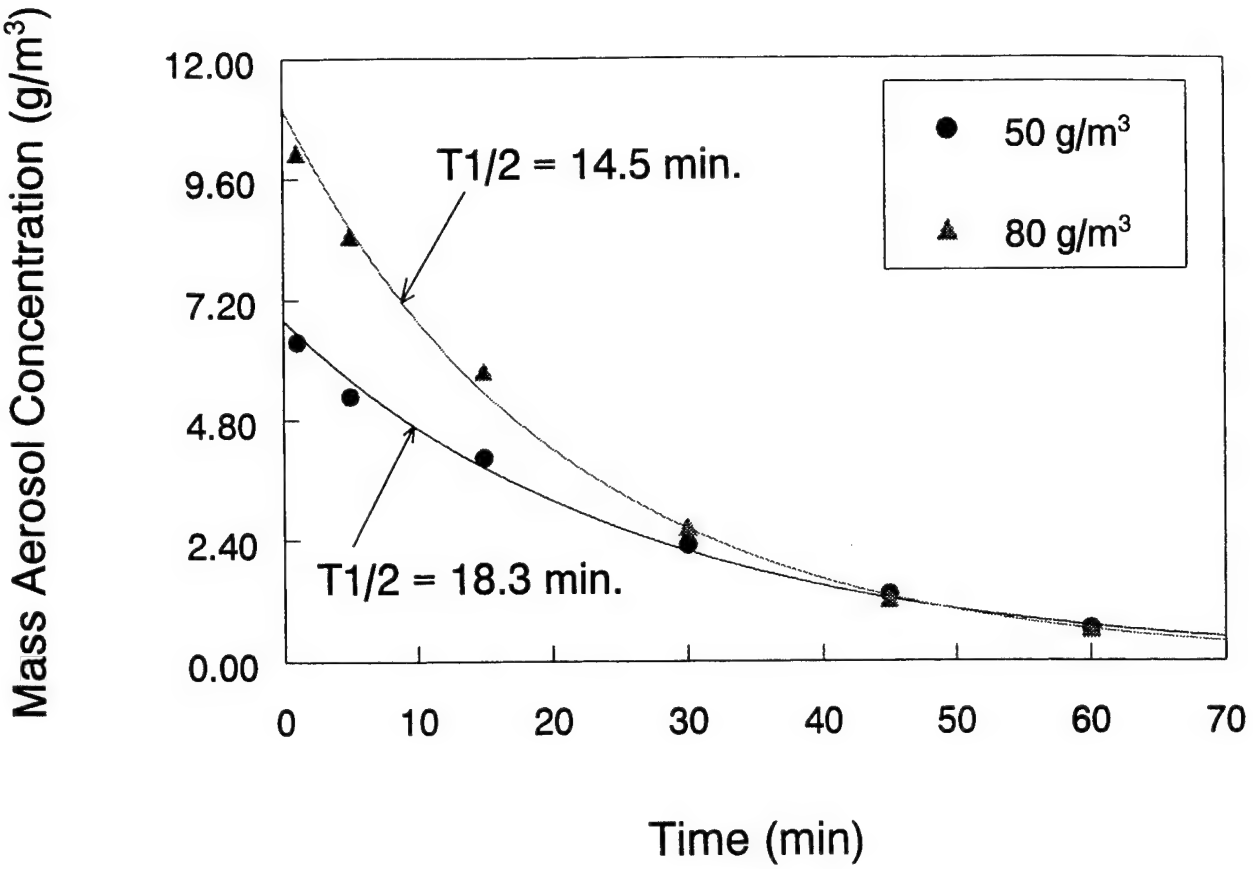


Figure 1. Exponential decay of SFE-A at loads (nominal concentrations) of 50 and 80 g m^{-3}.

for an SFE load of 80 g m^{-3} (Fig.1). The particle size (MMAD) and particle size distribution (σg) ranged from 2.08 to 2.49 mm and from 1.54 to 1.93, respectively (Table 2). In general, the aerosol by-products of SFE had a tendency to grow in size over time, most likely due to agglomeration (Fig. 2).

Clinical observations

No deaths were reported during the study. Animals exposed to the by-products of SFE exhibited signs of dyspnea, lack of coordination, lethargy and coughing/sneezing. Head pulling or straining was observed frequently, i.e. the animal would extend the head back, up and away from the body. As loads and length of exposure increased, these signs became more pronounced. All animals appeared to recover after being placed in fresh air. Animals exposed to an SFE load of 80 g m^{-3}, regardless of exposure length, exhibited a yellow viscous material around the nasal area.

Table 2. Mean and SEM for mass median aerodynamic diameter (MMAD; μm) and particle size distribution (σg) after the pyrolyzation of SFE-A at nominal loads of 50 and 80 g m^{-3}

Nominal load		1 min	15 min	60 min
50 g m^{-3}	MMAD	2.1 ± 0.1	2.6 ± 0.1	2.6 ± 0.1
	σg	1.66 ± 0.10	1.58 ± 0.06	1.74 ± 0.04
80 g m^{-3}	MMAD	2.0 ± 0.1	2.6 ± 0.1	2.6 ± 0.1
	σg	1.66 ± 0.10	1.58 ± 0.06	1.74 ± 0.04

Postmortem

No lesions were noted on the trachea, lung, heart or abdominal organs after gross examination. However, trace amounts of dried reddish-brown material were found around the nares in 100% of the animals exposed to an SFE load of 80 g m^{-3} for 60 min (1 h and 24 h post-exposure) and an SFE load of 80 g m^{-3} for 15 min (14 days post-exposure).

Histopathology

Lesions were noted in the nasal turbinates and were limited to those animals exposed to an SFE load of 80 g m^{-3}. The percentages of affected animals per post-exposure time points are as follows: 100% at 1 h, 80% at 6 h, 100% at 24 h, 0% at 7 days and 0% at 14 days. Lesions consisted of mild to minimal multifocal necrosuppurative rhinitis, primarily in the respiratory epithelium of the ventro-distal turbinates. There was also some minimal necrosis of the olfactory epithelium in the ethmoid turbinates (Fig. 3). Edema was noted in the lungs and limited to those animals exposed to an SFE load of 80 g m^{-3} (Fig. 4). The percentage of animals with mild to moderate edema per post-exposure time points are as follows: 40% at 1 h, 80% at 6 h, 60% at 24 h, 0% at 7 days and 0% at 14 days. One animal in the 6 h post-exposure group had severe

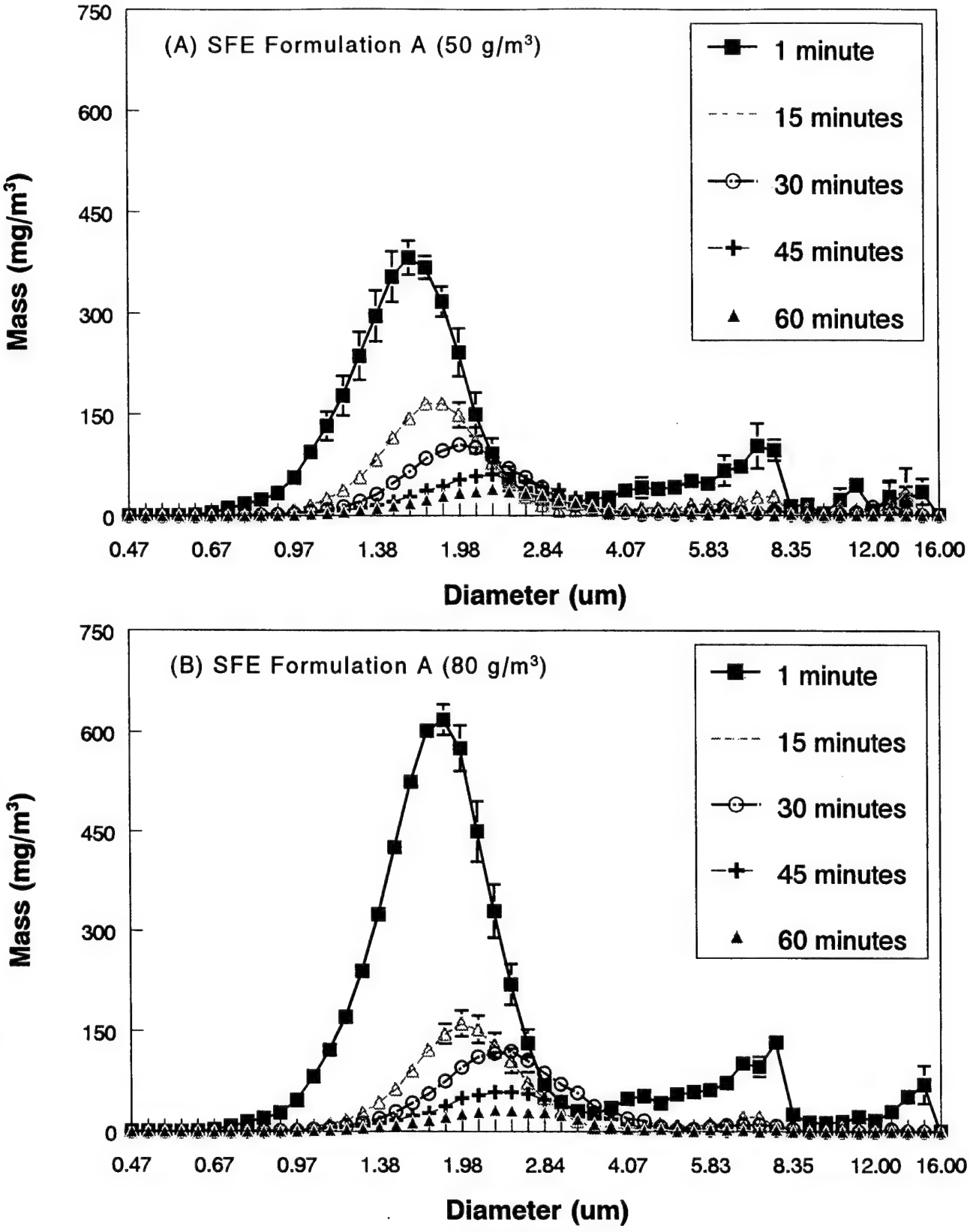
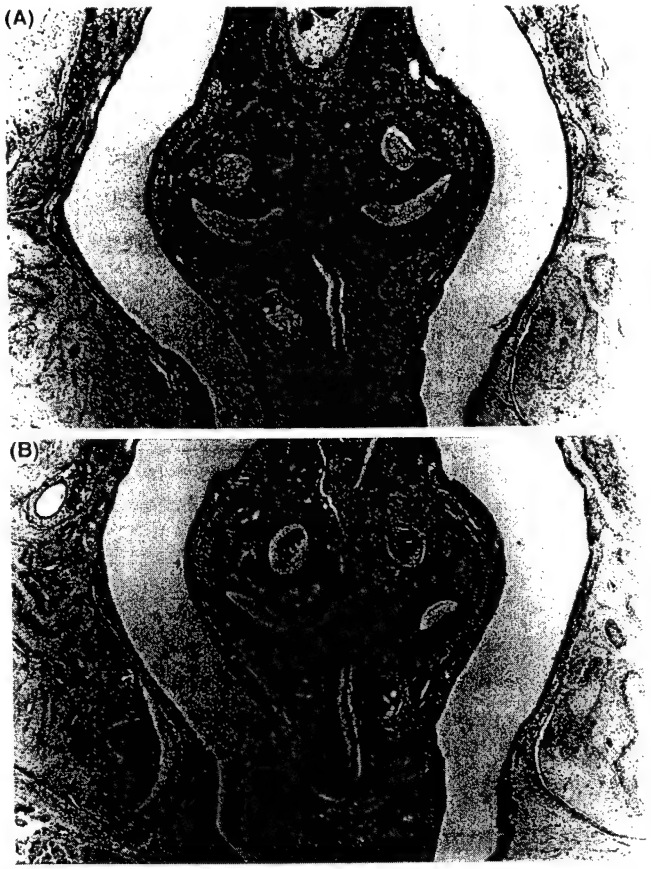


Figure 2. Spectrex Fire Extinguishant Formulation A particle size change as a function of time for loads (nominal concentrations) of 50 g m^{-3} (A) and 80 g m^{-3} (B). The *x*-axis (diameter) is non-linear, therefore sizes are midpoint of variable range size bin. The *y*-axis consist of values calculated from particle mass deposited per bin by the APS distribution program.



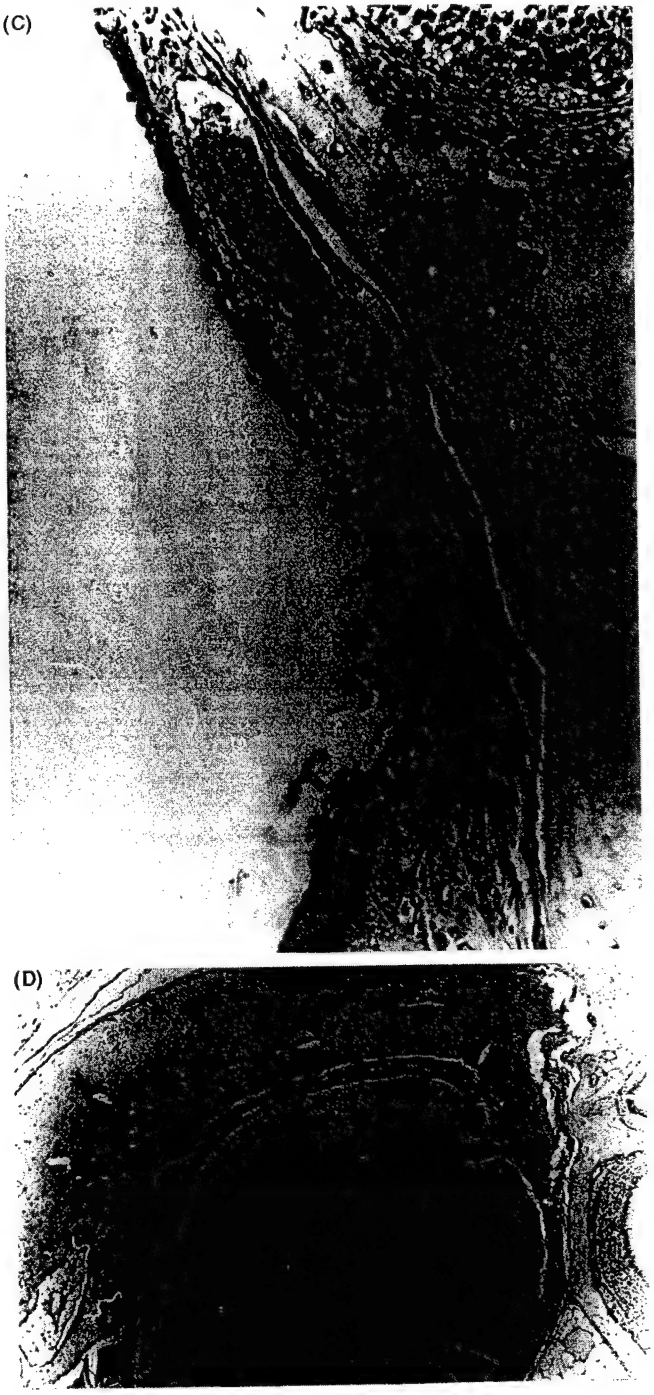


Figure 3. Histopathology of rat nasal turbinates exposed to an SFE load of 80 g m^{-3} for 60 min. (**A**) A 10× image of distal nasal turbinates: control animal. Demonstrates normal appearance of respiratory epithelium. (**B**) A 10× image of distal nasal turbinates of a Fischer 344 rat 1 h after exposure to an SFE load of 80 g m^{-3} for 60 min. Demonstrates multifocal minimal erosion and necrosis of respiratory epithelium, where it adjoins squamous epithelium. This is characterized by pyknosis of nuceli, condensation of cytoplasm and sloughing of cells. (**C**) A 16× image of distal nasal turbinates of a Fischer 344 rat 6 h after exposure to an SFE load of 80 g m^{-3} for 60 min. There is necrosis and erosion of respiratory epithelium, with multifocally dense infiltrates of neutrophils admixed with cellular debris within submucosa. Note: Cellular debris admixed with proteinaceous exudate within nasal passage. (**D**) A 10× image of distal nasal turbinates of a Fischer 344 rat 7 days after exposure to an SFE load of 80 g m^{-3} for 60 min. Respiratory epithelium is normal, demonstrating mild transitory nature of the toxic response. (**E**) A 33× image of slide (D). (**F**) A 10× image of distal nasal turbinates of a Fischer 344 rat 7 days after exposure to an SFE load of 80 g m^{-3} for 60 min. Respiratory epithelium is normal, demonstrating the mild transitory nature of the toxic response.

Figure 3. Continued.

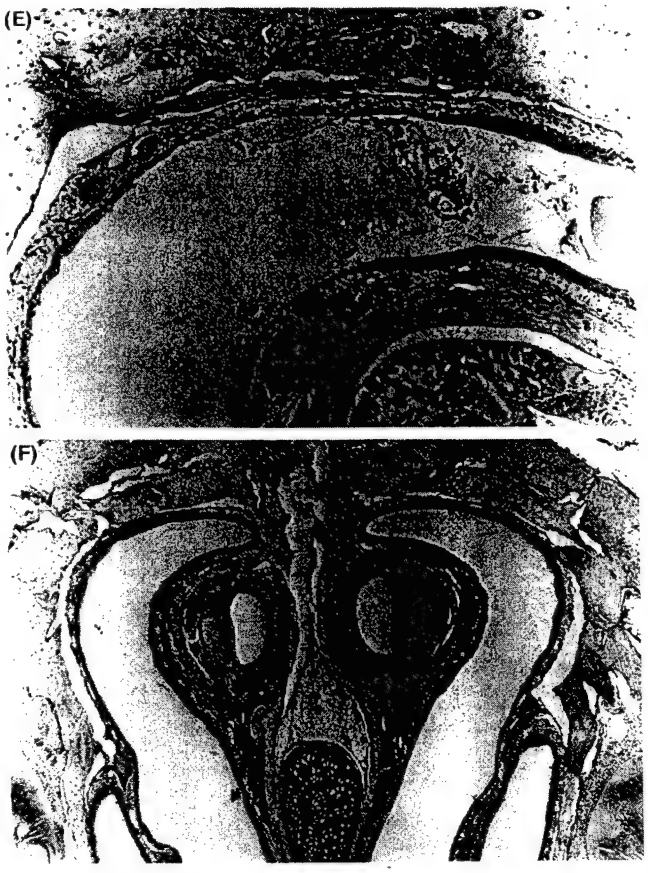
edema. In addition to edema, moderate to severe pneumonia was present in 40% of the animals at the 24-h post-exposure time point and in 20% of the animals at the 7-day post-exposure group. The pneumonia consisted of multifocal to occasionally coalescing foci of alveoli filled by edema fluid and occasional hemorrhage, with numerous macrophages admixed with lesser lymphocytes, plasma cells, neutrophils and cellular debris.

Wet/dry weight determination

There was no significant difference between the control groups and the exposure groups for the percentage of water in the lung, the weight (g) of water in the lung per kilogram of body weight and the weight (g) of solid (dehydrated) lung per kilogram of body weight. However, there was an increase (~10%) in the percentage of water in the lung for animals exposed to an SFE load of 80 g m^{-3} for 60 min, but this was not statistically significant.

Enzyme analysis

Total protein was elevated in BAL for animals exposed to an SFE load of 50 g m^{-3} for 60 min at post-exposure time points of 6 and 24 h, and significantly elevated ($P = <0.01$) for animals exposed to an SFE load of



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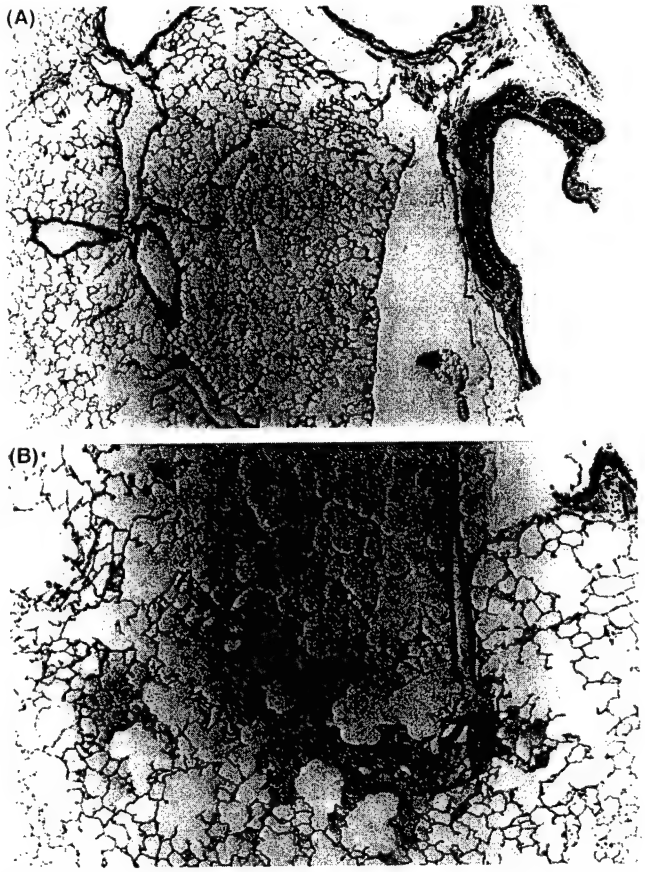

Figure 4. Histopathology of rat lungs exposed to an SFE load of 80 g m^{-3} for 60 min. (**A**) A 10× image of lung: control animal. Demonstrates normal appearance of respiratory epithelium. (**B**) A 16× image of a lung from a Fischer 344 rat 1 h after exposure to an SFE load of 80 g m^{-3} for 60 min. Demonstrates mild multifocal alveolar edema. Alveolar lumina are filled with pale proteinaceous material. (**C**) A 40× image of slide (B). At higher magnification, there are a scattered number of macrophages within edematous alveoli. (**D**) A 10× image of a lung from a Fischer 344 rat 6 h after exposure to an SFE load of 80 g m^{-3} for 60 min. Demonstrates moderate multifocal to coalescing alveolar edema. (**E**) A 40× iamge of slide (D), demonstrating the extensive nature of the edema. (**F**) A 10× image of a lung from a Fischer 344 rat 7 days after exposure to an SFE load of 80 g m^{-3} for 60 min. Normal lung, demonstrating the transient nature of the lesion.

80 g m^{-3} for 60 min at post-exposure time points of 6 and 24 h (Fig. 5). Total protein was within its normal range for all remaining exposure groups. No alterations were noted in acid phosphatase, alkaline phosphatase, lactate dehydrogenase and β-glucuronidase activity of BAL.

DISCUSSION

The generation of the SFE aerosol required modification of the procedures normally used to investigate the acute inhalation toxicity of test agents. During the pyrolyzation process, SFE is capable of producing temperatures over 1000°C as well as a pressure pulse during initiation.[6] Because of this physical phenomenon, the generator was placed outside the exposure system and connected to the chamber via an aluminum duct. This allowed for the dissipation of both the thermal energy and the pressure pulse.

Spectrex Fire Extinguishant produces its fire-extinguishing capabilities through a single-pulse generation of a dry-powder aerosol. To evaluate the toxicity of the aerosol under normal use conditions, the chamber was operated in static mode. Because of the static mode, an exponential decay of aerosol concentration was noted over the exposure duration. The initial aerosol concentration for loadings of SFE at 50 and 80 g m^{-3} was uniquely different, but converged to approximately the same concentration by 60 min (Fig. 1). The dynamic behavior of the aerosol concentration obfuscates the determination of an exposure–response relationship.

Dynamic changes in aerosol size distribution showed a tendency for the MMAD to increase over time despite early settling of larger particles. This shift in particle size can be attributed to agglomeration phenomena (i.e. the high initial particle concentration) and hygroscopic growth of individual particles. Considering only total lung deposition, the shift in aerosol size distribution is not considered to be significant. However, an increase in aerosol size distribution may cause large shifts in regional pulmonary deposition patterns. Despite the increase in aerosol size distribution, the MMAD of the aerosol population remained 2–3 μm in diameter, suggesting a preference for deposition in th distal airways and alveolar spaces.

Clinical observation of cyclic hyper/hypoventilation breathing patterns, sneezing or coughing suggests that inhalation of SFE aerosol is irritating to the mucous membranes of the nasal cavity and respiratory tract. It

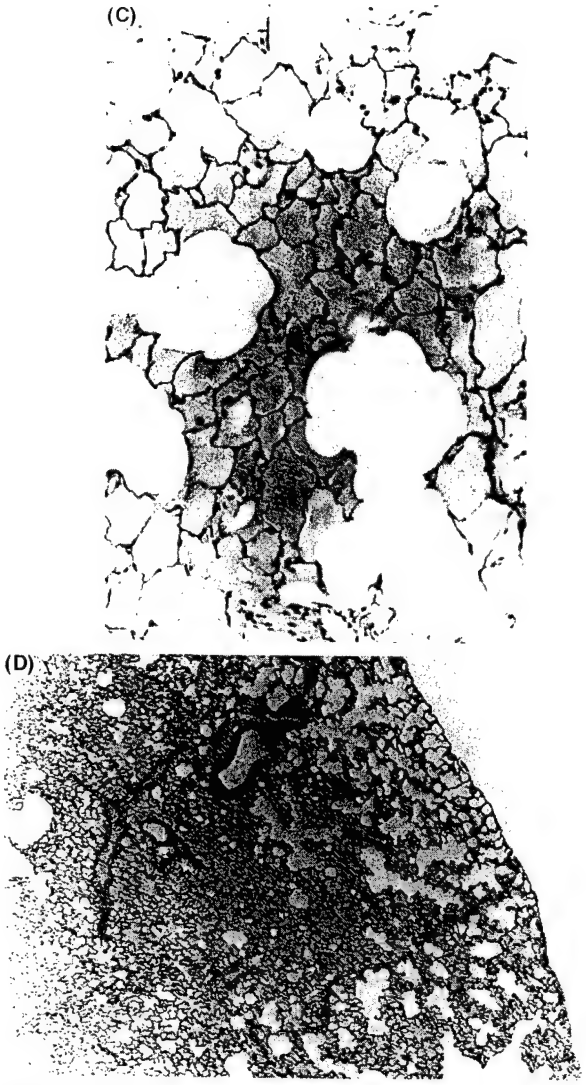

Figure 4. Continued.

has also been shown that after pyrolyzation of SFE the concentrations of carbon dioxide (CO_2) and carbon monoxide (CO) within the chamber are elevated, subsequently increasing the pCO_2 and carboxyhemaglobin levels in the test animals, respectively.[7] Both elevated pCO_2 and reduced pO_2 (via the formation of carboxyhemaglobin) will stimulate ventilation.[11] In an SFE environment, an increase in the rate of ventilation would increase the deposition of the aerosol in the lung and the potential for pulmonary insult. The results of this study showed a time- and dose-dependent hydrostatic pulmonary edema. The data support this conclusion based upon the elevated protein levels in bronochoalveolar lavage with no accompanying increase in enzyme activity. An increase in enzyme activity would suggest significant tissue or cellular damage and cell death. This would classify the results as permeability pulmonary edema. A few of the animals developed severe pulmonary complications resulting in apparent tissue damage, particularly in the nasal mucosa. Histological examination demonstrated pulmonary tissue damage in some of the animals, as evidenced by cellular debris in alveolar spaces and persistent pneumonitis. Although the mechanism of hydrostatic edema is not clearly understood, it is speculated that rapid dissolution of potassium chloride aerosol would produce a local perturbation in the electrolyte balance. This balance is important in regulating the fluid homeostasis of the lung.[12] Changes in hydrostatic pressure will lead to compartmental shifts of tissue fluids, resulting in interstitial edema.[13] Therefore, in severe situations hydrostatic edema can progress to frank alveolar edema.[14]

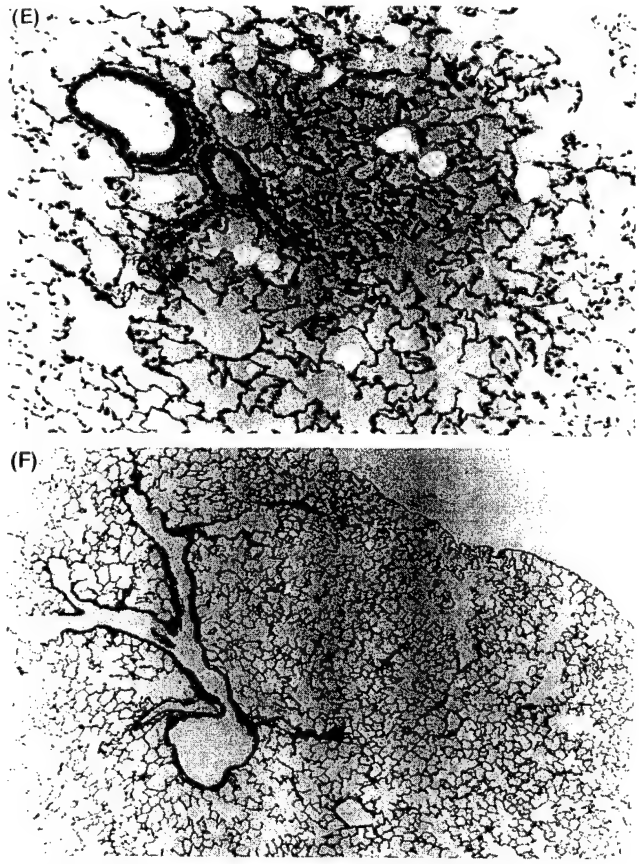

Figure 4. Continued.

CONCLUSION

The pyrolyzation of SFE results in the formation of a potassium chloride aerosol. The aerosol is subject to agglomeration and yet remains of a respirable size. Exposure to the resulting aerosols from the pyrolysis of SFE-A produced hydrostatic pulmonary edema and olfactory necrosis. These observations were limited to only those animals exposed at a loading equivalent of 80 g m^{-3} for 60 min. The pulmonary edema is most likely the result of hydrostatic effects. This is based upon the evidence of an increase in protein in the BAL fluid with no subsequent increase in cytosolic enzyme levels. The hydrostatic pulmonary edema and olfactory necrosis were resolved by 7 days post-exposure.

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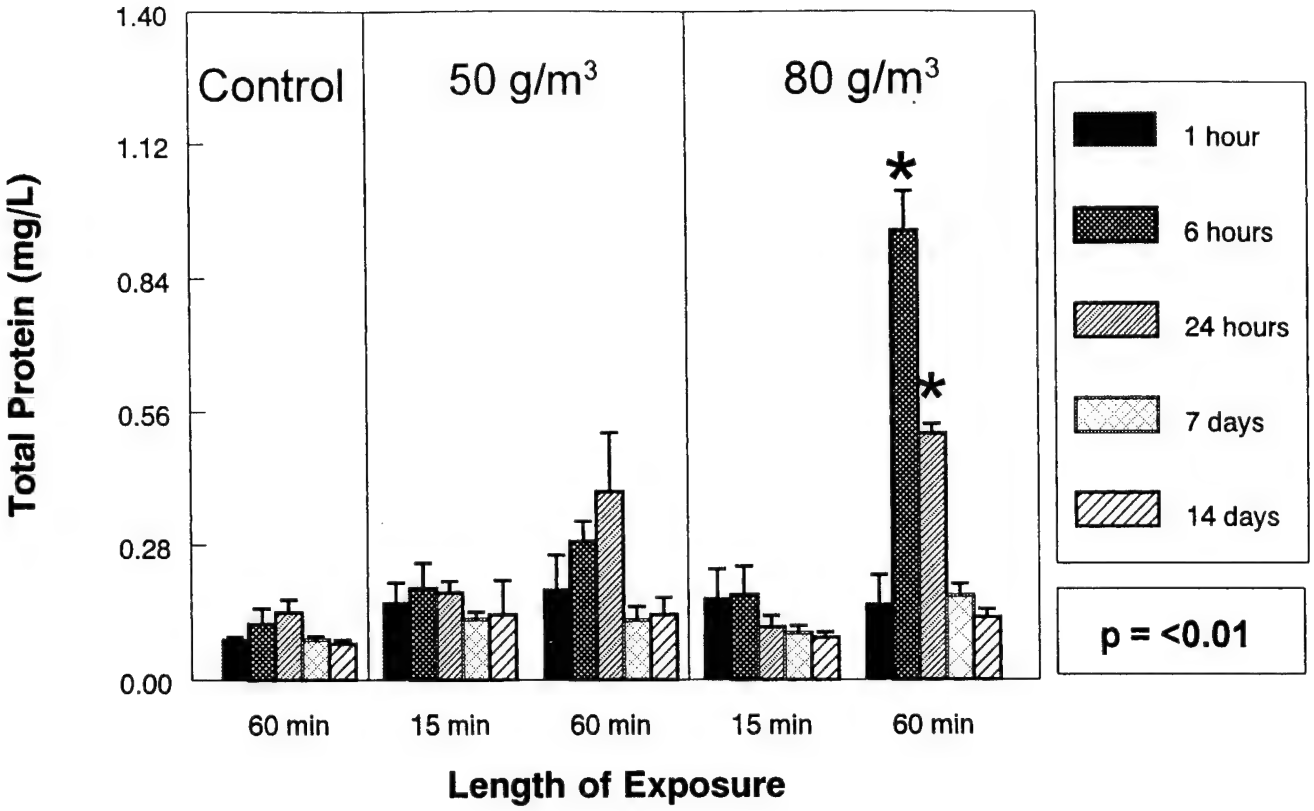

Figure 5. Total protein analysis in BAL of animals exposed to pyrolyzed SFE-A ($P = <0.01$).

Disclaimer

The opinions expressed herein are those of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval service at large. The experiments reported herein were conducted according to the principles set forth in the 'Guide for the Care and use of Laboratory Animals', prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, DHHS, National Institute of Health Publication 85-23, 1985, and the Animal Welfare Act of 1966, as amended.

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PRELIMINARY ASSESSMENT OF A PYROTECHNICALLY GENERATED AEROSOL FIRE SUPPRESSANT

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Fischer 344 rats (approximately 250–300 g) were exposed to the aerosol resulting from the pyrolysis of SFE Formulation A at a loading of 50 or 80 g/m³. Animals were exposed for 15 or 60 min and euthanized 1 h postexposure. The exposures were conducted in a 700-L inhalation chamber under static conditions. Aerosol samples were collected and analyzed for mass concentration and size distribution (mass median aerodynamic diameter, MMAD, and geometric standard deviation, σ_g). Lungs were collected for wet/dry weight ratio analysis, and nasal turbinates were prepared for histopathology examination. No deaths occurred during the study or postexposure observation period. Animals exposed to SFE exhibited signs of dyspnea, lack of coordination, and lethargy. As the load and exposure length increased, these signs became more pronounced. No lesions were noted in the trachea, lung, heart, and abdominal organs upon gross examination. No histopathological abnormalities were noted in the nasal turbinates. Changes in blood gases, blood pH, the various hemoglobin types, and serum glucose were noted. Of these changes, carboxyhemoglobin was the most significant, with an increase of 40%. The remaining serum chemistry parameters evaluated were within their respective biological ranges. Wet/dry lung ratio showed no difference between control and exposure groups.

Chlorofluorocarbons (CFCs) and halocarbons (halons) are commonly used for fire suppression. During the suppression of a fire, CFCs and halons are unintentionally released into the environment. The release of such fluorocarbons has been shown to indirectly destroy atmospheric ozone (Cicerone, 1994). Because of this destructive effect, countries around the world have agreed to slowly phase out the production (1987 Montreal Protocol) and eventual use of CFCs and halons. With

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the impending halt, fire extinguishant manufacturers have begun to investigate alternative forms of fire suppression, such as dry-powder aerosol fire suppressants.

Dry-powder aerosols are used primarily in various hand-held fire extinguishers. These extinguishers act as streaming agents/devices and propel the aerosol onto the fire. The aerosol suppresses the fire by various mechanisms (i.e., vaporization, decrepitation, decomposition, and surface mediated phenomena), physical properties (i.e., mass concentration, size distribution, and specific surface area, SSA), and chemical properties (i.e., particle density, morphometry, and chemical composition) of the aerosol (Sheinson et al., 1993; Freidman, 1993; Ewing et al., 1989, 1992). In order for these extinguishers to be effective, the aerosol particles must be large enough to achieve the proper momentum needed for an acceptable throwing distance. These aerosols are tens to hundreds of micrometers in diameter. Therefore, the inhalation hazard associated with the use of these extinguishers is minimal. However, the current trend in aerosol fire suppression is to reduce aerosol particle size. One such process capable of producing aerosol below 10 μm in diameter is the pyrotechnically generated aerosol system. Spectrex fire extinguishant (SFE) is an example of a pyrotechnically generated aerosol.

Pyrotechnically generated aerosols are formed when the parent (or starting) material reaches an initiation temperature. In the case of SFE, this temperature is ~500°C. Once the initiation temperature is reached, the parent material undergoes a cascade of chemical reactions that ultimately results in the production of the aerosol. SFE Formulation A is comprised primarily of potassium perchlorate, which upon decomposition produces an aerosol of potassium chloride.

When determining the toxicity of an aerosol, its physical and chemical composition must be evaluated. For SFE, the aerosol produced during the pyrolyzation (i.e., evaporation/condensation) process typically is of a respirable size, that is, 1–10 μm in diameter. These particles when inhaled are capable of penetrating deep into the tracheobronchial tree (Hatch & Gross, 1964). Therefore, particle size is important in determining the toxicity of an aerosol.

The specific surface area and chemical solubility of the particulate material are also important in determining the toxicity of aerosols. As is true of other administration routes, the lung's response to dissolved material depends highly on the chemical composition of the particulate. Particulates that are a salt or have a high pH may irritate the lung and cause imbalances in hydrostatic forces, thereby altering pulmonary function. Direct dermal, ocular, or nasal contact with the aerosol may also result in irritation at these sites. During the pyrolyzation process, pyrotechnically generated aerosols may also release combustion gases, such as carbon monoxide. Such an exposure could alter gas exchange, with disorientation and incapacitation being the

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outcome at sufficiently high doses. Therefore, due to the complex nature of the end products of pyrotechnically generated aerosols, toxicity determination must take into account the physical and chemical aspects of the aerosol. The objective of this study was to first characterize the aerosol atmosphere of SFE Formulation A as a function of time and then to assess its acute toxicity (i.e., survivability).

METHODS

Chemicals

SFE Formulation A was supplied by Spectronix Ltd. (Tel Aviv, Israel).

Inhalation Chamber Configuration and Operation

The exposure system consisted of a modified Hinners-type 700-L inhalation chamber with a supply/exhaust system, a specifically designed aerosol generator, and an exhaust scrubber (Kimmel & Yerkes, 1990). The aerosol generator was placed outside the exposure chamber because of the pyrotechnic nature of the parent material. To transport aerosol into the chamber, the system was operated in the dynamic mode until the generation of aerosol had ceased. Once filled, pneumatic valves transformed the chamber to static operation. The test atmosphere generator consisted of two flanged 4-in sections of schedule 80 stainless steel pipe bolted together. An 1/8-in thick sintered stainless steel plate was located between the 2 sections of pipe. Air entered the generator through the lower chamber and passed through the sintered plate into the ignition chamber. The ignitor consisted of a 6-cm piece of 26-gauge nichrome wire attached to insulated copper electrodes, and placed through the wall of the upper (ignition) chamber. The nichrome wire was coiled to fit in the bottom of a ceramic combustion boat placed on the sintered plate. A 6-A current was passed through the ignitor using an 18-V source to produce a temperature of 550–600°C. A thermocouple was placed 5 cm above each SFE pellet to monitor the ignition and combustion temperatures. Aerosol samples were collected from sampling ports located in the rear of the chamber and positioned within the "breathing zone" of the chamber. The aerosol was analyzed for mass concentration and particle size distribution. The system was exhausted through a scrubber at the conclusion of each exposure.

Exposure Atmosphere Characterization

The exposure aerosol mass concentration was determined using filter samples. Samples were collected on 47-mm Gelman 61631 A/E glass fiber filters that were stored in a desiccator prior to use. Filters

were weighed on a Cahn C-31 microbalance (Fisher, Cincinnati, OH) and placed in a brass filter holder (IN-TOX Products, Albuquerque, NM). Samples were collected at 1, 5, and 15 min for 15-min exposures and 1, 5, 15, 30, 45, and 60 min for 60-min exposures. The flow rate through a filter was 5 L/min, with a sampling time of 15 s.

Particle size distribution was determined using a cascade impactor (IN-TOX Products, Albuquerque, NM). The impactor designs were based on Marple's criteria (Marple, 1978). Aerosol particles were collected on 37-mm stainless steel substrates coated with Apiezon grease to minimize particle bounce. A 47-mm Gelman 61631 A/E glass fiber filter was used as a final filter. Substrates and filter were weighed on the Cahn C-31 microbalance. Samples were collected at the beginning and end of each exposure. The flow rate through the impactors was 20 L/min with a sampling time of 4–15 s. Because of the dynamic nature of the aerosol, aerosol size distribution was also determined with a TSI model 3300 aerodynamic particle size analyzer (APS; TSI Corp., St. Paul, MN). The APS measured article number as a function of mass weighted aerodynamic diameter. Two TSI model 3302 diluters, each with a 100:1 dilution probe, proceeded the APS, providing a final dilution of 10,000:1. The real-time aerosol analysis was performed on a Zenith data system 286 PC with TSI model 390041 APS Advanced Software, version B, using a density value of 2 g/cm^3 (bulk density of KCl 1.98). Samples were collected for 10 s at 1, 15, 30, 45, and 60 min.

Oxygen was measured on a Teledyne analytical instrument percent oxygen analyzer (model 326RA, Teledyne Analytical Instruments, City of Industry, CA). Carbon dioxide and carbon monoxide were measured with Beckman industrial model 865 infrared analyzers (Beckman Instruments, La Habra, CA). Samples were collected at 1, 5, 15, 30, 45, and 60 min, depending on the length of the exposure.

Animals

Twenty male Fischer CDF (F-344)/CrlBR rats weighing between 200 and 250 g were purchased from Charles River Breeding Labs (Wilmington, MA), and were provided Formula lab chow 5008 (Purina Mills, Inc., St. Louis, MO) and reverse-osmosis-filtered water ad libitum. The rats were kept on a 12-h light/dark cycle and acclimated to their environment for 2 wk prior to use.

Experimental Design

Animals were randomized into five exposure groups of four animals each. Exposures were performed in the previously described 0.7-m^3 whole-body inhalation chamber. Exposure groups are given in Table 1.

TABLE 1. Exposure groups

Group	Nominal concentration	Length of exposure
1	Control	60 min
2	50 g/m^3 of Formulation A	15 min
3	80 g/m^3 of Formulation A	15 min
4	50 g/m^3 of Formulation A	60 min
5	80 g/m^3 of Formulation A	60 min

Clinical Observations, Postmortem, Histopathology, and Blood Collection

Animals were observed throughout the duration of the exposure period. At the conclusion of each exposure, animals underwent an ocular and dermal irritation examination prior to euthanasia. Animals were euthanized 1 h postexposure by intraperitoneal injection of a euthanasia mixture consisting of ketamine HCl (Vetalor; Parke-Davis, Morris Plains, NJ) and xylazine (Rompun; Mobay Corporation, Shawnee, KS) at a mix ratio of 7 ml to 3 ml, respectively. Once euthanized, the abdominal and thoracic cavities were opened and blood samples were collected from the left ventricle of the heart with a 10-cm^3 syringe containing heparin. A portion of each blood sample was transferred to a 3-ml Vacutainer containing heparin (green top) for serum chemistry, while the remaining portion was analyzed for blood gases and pH. Gross examination was performed on the trachea, lung, heart, and abdominal organs. The trachea and lung were removed from the thoracic cavity and trimmed for wet/dry weight determinations. The head was removed and transversely cut at the level of the incisive papilla and second palatal ridge using a Buehler Isomet low-speed saw with diamond wafering blade (Evanston, IL) to examine nasal turbinates. Each nasal turbinate section was placed in 10% neutral buffered formalin and decalcified for 3 days in 10% solution of ethylenediamine tetraacetic acid (EDTA; Sigma, St. Louis, MO). The nasal turbinates were processed for histological examination (light microscopy). Each section was embedded in paraffin, sectioned at 3–4 μm, and stained with hematoxylin and eosin.

Ocular and Dermal Irritation Examination

Ocular and dermal examinations were conducted pre-, and postexposure. To evaluate dermal irritation, a 2 cm by 6 cm strip was shaved on the dorsal side of all animals with clippers approximately 24 h prior to the exposure. The dermis of each animal was scored before and after each exposure (Draize, 1959). To evaluate ocular irritation, a solution of fluorescein (2% fluorescein in phenyl mercuric nitrate) was applied to each eye of the animal and observed under

an ultraviolet (UV) light. The corneal opacity was scored before and after each exposure (Draize & Keller, 1944).

Blood Gas, pH, Hemoglobin, and Serum Chemistry Analysis

Blood gases and pH were determined on a Ciba-Corning 288 blood gas analyzer (Corning Diagnostics Corp., Medfield, MA). Blood gas parameters were blood pH, and partial pressures of oxygen, carbon dioxide, and bicarbonate. Hemoglobin analysis was performed on a Ciba-Corning 2500 CO-oximeter (Corning Diagnostics Corp., Medfield, MA). Hemoglobin parameters were total hemoglobin, carboxyhemoglobin, methemoglobin, oxyhemoglobin, and deoxyhemoglobin. Serum chemistries were performed on a Kodak Ektachem 700 analyzer (Rochester, NY). Serum chemistry parameters were glucose, sodium, potassium, chloride, calcium, magnesium, and phosphorus.

Wet/Dry Lung Weight Determinations

Wet/dry lung ratios were measured by the method of Staub (1974), with minor modifications. After the animals were euthanized, the thoracic cavity was opened to expose the lung, heart, and cervical trachea. The tissue between the thoracic and cervical trachea was excised. The trachea was ligated 1–2 mm below the pharynx. After transection of the aorta and vena cava the heart was excised, the lung extracted en bloc, and the esophagus removed. The lungs were rinsed with saline and blotted dry with gauze pads. A small, preweighed S-shaped hook was inserted through the trachea just above the ligature and lung wet weight recorded. The lung was then suspended from a drying rack via the S-hook and placed in a drying oven at 110°C for 24 h. At the conclusion of 24 h, the lung dry weight was recorded. Corrected lung wet weight (WW) and dry weight (DW) were determined by subtracting the weight of the S-hook. Pulmonary edema formation was quantified by the difference between the exposed and control groups measuring for percent lung water (H_2O); grams water in the lung per kilogram body weight (BW); and grams solid (dehydrated) lung per kilogram body weight.

Statistical Analysis

A two-factorial analysis of variance with Bonferroni multiple comparison was performed on all blood gases, pH, hemoglobin, serum chemistry, and wet/dry lung weight parameters. The equality of variance was tested using Levene's test.

RESULTS

Exposure Atmosphere

Since the SFE aerosolization process required ignition of a solid pellet, it was necessary to design an exposure system that would dis-

sipate any pressure pulse and/or thermal energy created during the pyrolyzation. A specified load of SFE Formulation A (g SFE/volume of exposure chamber) was ignited at the start of the exposure period and the resulting aerosol trapped within the inhalation chamber. No additional SFE Formulation A aerosol was added to the chamber during the exposure. The chamber was operated under subambient pressure at the start of each exposure. A pressure pulse was observed 10–15 s after ignition and lasted for 5–10 s; the chamber pressure never exceeded 5 cm H_2O. This pressure pulse dissipated through the chamber inlet and exhaust system since the chamber was operating in the dynamic mode. Chamber inlet and exhaust valves were closed after dissipation of the pressure pulse as the aerosol reached maximal concentration. Temperatures within the generator were approximately 1025°C for a 50 g/m^3 load and 1200°C for an 80 g/m^3 load, but the chamber's interior temperature remained at ambient temperature (22–26°C) for the duration of the exposure. The chamber was successfully operated under static conditions; no supplemental oxygen was required to maintain acceptable oxygen partial pressure.

Aerosol concentrations determined from filter measurements are shown in Figure 1. The average initial aerosol concentration was 6.58 and 8.40 g/m^3 for an SFE load of 50 and 80 g/m^3, respectively. However, by the end of the exposure period, the aerosol concentration was approximately 0.86 g/m^3 independent of SFE load. The aerosol half-life was 20.7 and 16.7 min for an SFE load of 50 and 80 g/m^3, respectively. Aerosol MMAD as determined by cascade

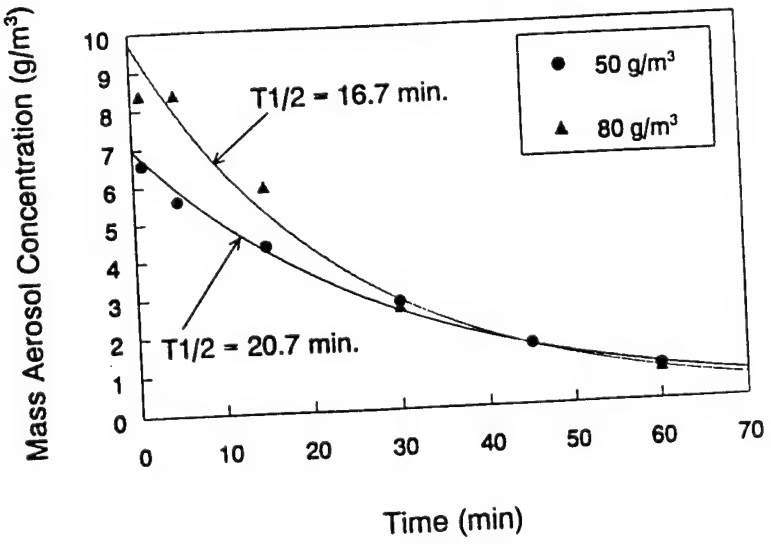

FIGURE 1. SFE Formulation A mass aerosol concentration.

impactor ranged from 1.94 to 2.54 μm and from 2.09 to 2.85 μm for an SFE load of 50 and 80 g/m³, respectively. The σ_g ranged from 1.4 to 1.8 and from 1.5 to 2.1 for an SFE load of 50 and 80 g/m³, respectively.

The optical particle size analyses shown in Figure 2 illustrate the

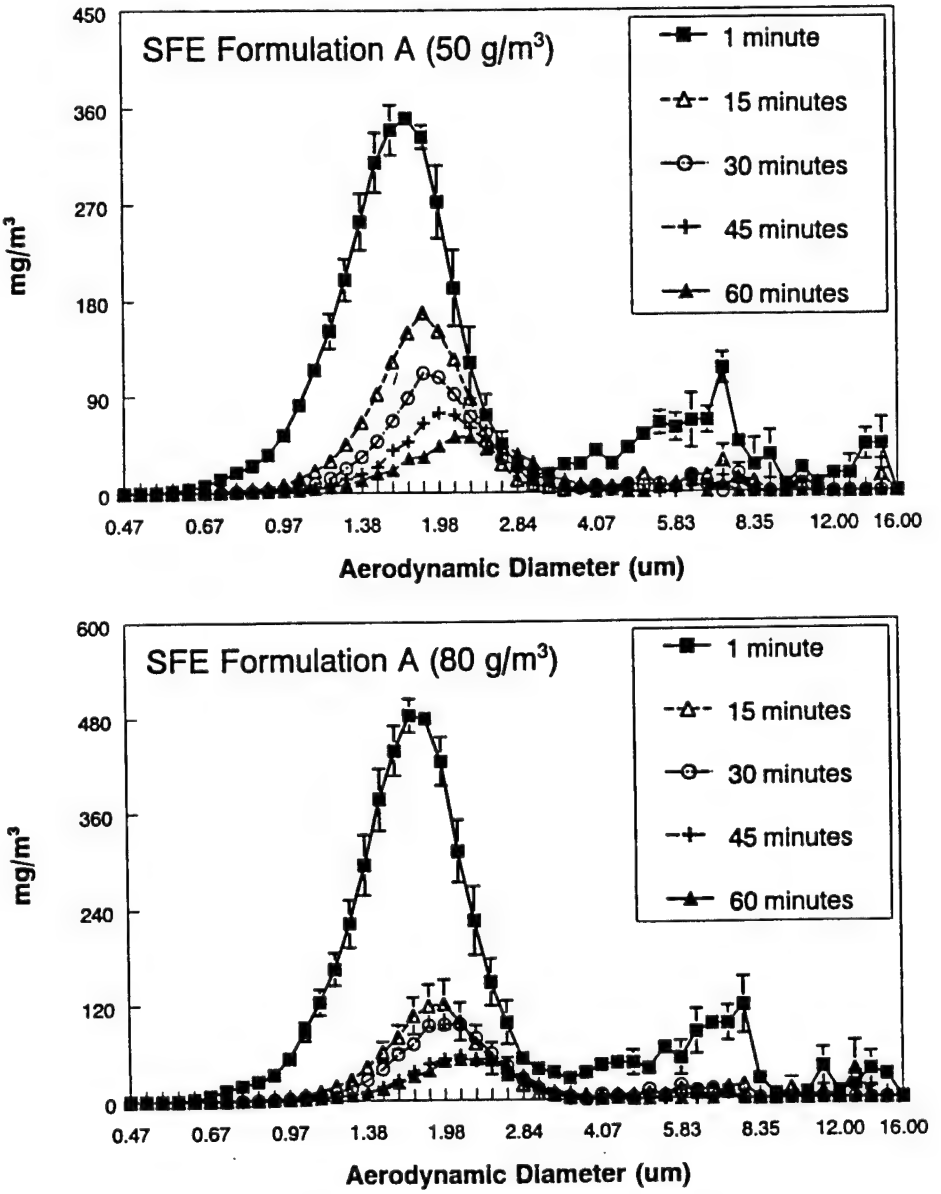

FIGURE 2. SFE Formulation A particle size change as a function of time. The *x*-axis (diameter) is nonlinear. Therefore sizes are midpoint of variable range size bin. The *y*-axis consists of values calculated from particle mass deposited per bin by the APS distribution program. (a) 50 g/m³; (b) 80 g/m³.

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TABLE 2. Mean gas concentrations after the pyrolyzation of SFE Formulation A

SFE load (g/m^3)	Carbon dioxide (ppm)	Carbon monoxide (ppm)	Oxygen (%)
50	10,530	2658	18.5
80	9731	6675	18.3

observation that SFE aerosols were bimodal for both exposure concentrations. The exposure aerosols were dynamic with respect to both concentration and MMAD during the exposure interval. An SFE load of 80 g/m^3 produces a higher peak at 1 min than an SFE load of 50 g/m^3, but after 60 min of exposure time the aerosols have decayed to similar concentrations and distributions. These observations are consistent with the filter and impactor measurements described earlier.

After the results of blood gas analyses were obtained, gas concentrations for the two SFE loadings were measured in the chamber without animals present. These measurements were taken after the completion of the pyrolyzation process and are shown in Table 2. Carbon dioxide and carbon monoxide are both present after the pyrolization of parent SFE material. The introduction of SFE pyrolysis products into the exposure atmosphere did not reduce chamber oxygen level below 18%.

Clinical Observations

No deaths were reported during the study. Animals exposed to SFE Formulation A exhibited signs of dypsnea, and apneustic breathing, lack of coordination, and lethargy. As loads and length of exposure increased, these signs became more pronounced. All animals appeared to recover once placed in fresh air.

Pathology

No lesions were noted on the trachea, lung, heart, and abdominal organs after gross examination. The only lesions observed grossly were red foci around the hilar region of the lungs of all animals exposed to an SFE load of 80 g/m^3 for 60 min. Nasal turbinates exhibited no abnormalities under histopathological examination.

Ocular and Dermal Irritation

All animals were free of ocular or dermal irritation and abnormalities prior to exposure. Postexposure examination showed no ocular or dermal irritation in the animals.

Blood Chemistry

There was no specific trend for pCO_2 and it was not statistically different from control (Figure 3). The pO_2 decreased for all exposure

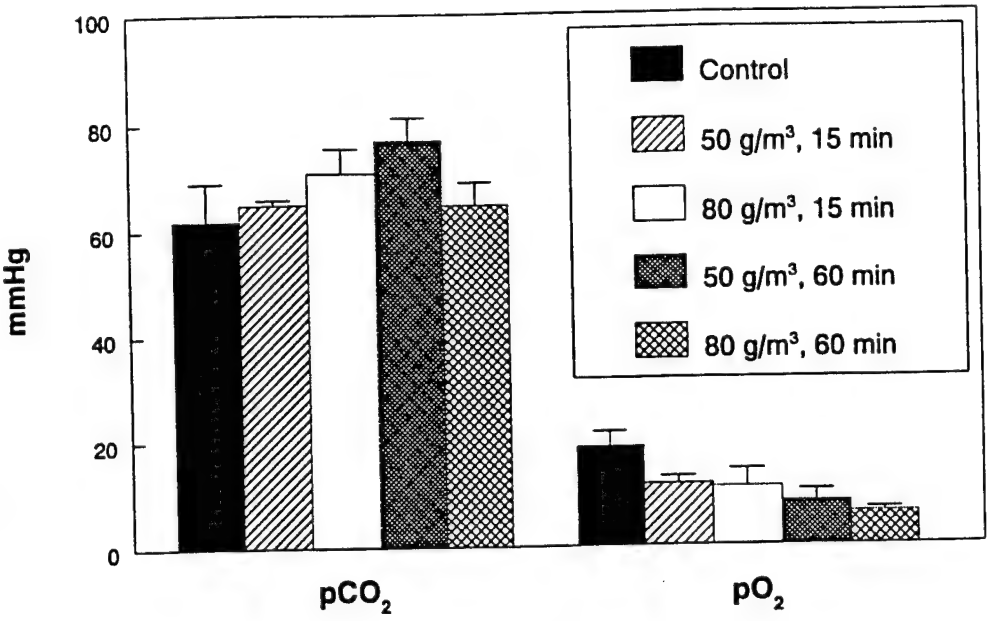

FIGURE 3. Partial pressure of carbon dioxide (pCO_2) and oxygen (pO_2) for Fischer 344 male rats exposed to the aerosol products of SFE Formulation A at load concentrations of 50 or 80 g/m³ for 15 or 60 min. Group 1 = control, group 2 = 50 g/m³ for 15 min, group 3 = 80 g/m³ for 15 min, group 4 = 50 g/m³ for 60 min, group 5 = 80 g/m³ for 60 min.

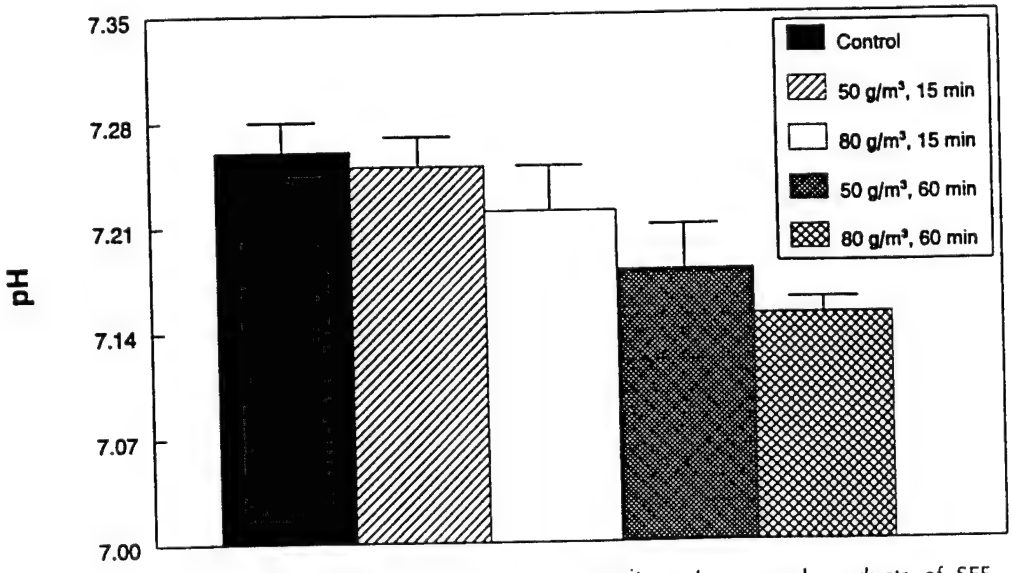

FIGURE 4. Blood pH for Fischer 344 male rats exposed to the aerosol products of SFE Formulation A at load concentrations of 50 or 80 g/m³ for 15 or 60 min. Group 1 = control, group 2 = 50 g/m³ for 15 min, group 3 = 80 g/m³ for 15 min, group 4 = 50 g/m³ for 60 min, group 5 = 80 g/m³ for 60 min.

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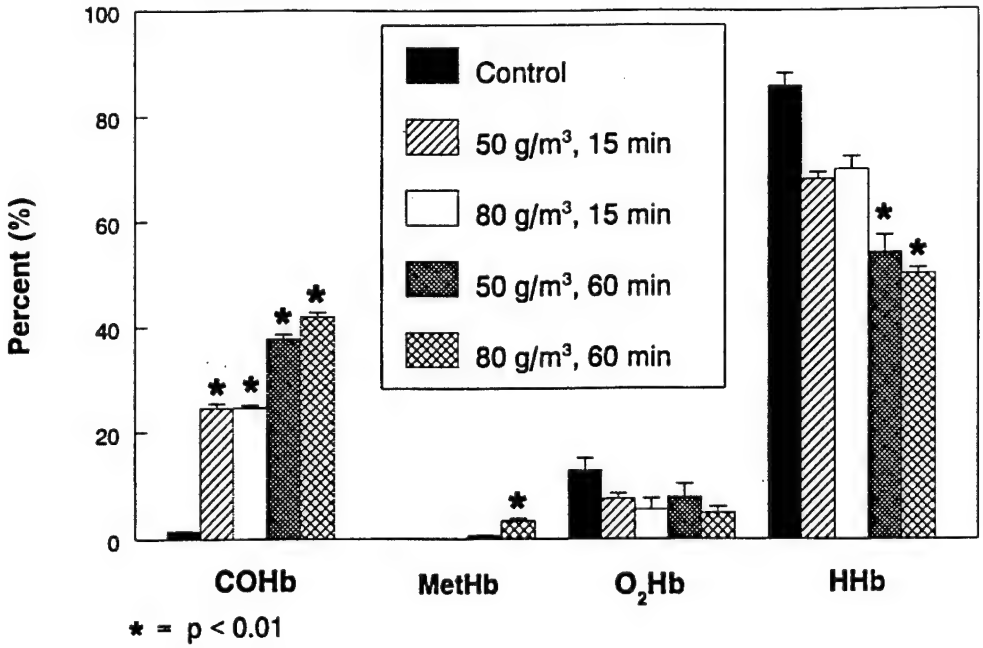

FIGURE 5. Hemoglobin analysis for Fischer 344 male rats exposed to the aerosol products of SFE Formulation A at load concentrations of 50 or 80 g/m³ for 15 or 60 min. Asterisk indicates significantly different than control at $p < .01$. Group 1 = control, group 2 = 50 g/m³ for 15 min, group 3 = 80 g/m³ for 15 min, group 4 = 50 g/m³ for 60 min, group 5 = 80 g/m³ for 60 min.

groups with increased loading and length of exposure (Figure 3), but the decrease was not statistically significant. Blood pH decreased for all exposure groups with increased load and length of exposure (Figure 4), but the decrease was not statistically significant.

Hemoglobin analyses are shown in Figure 5. Total hemoglobin was within its biological range. Carboxyhemoglobin was significantly increased ($p < .01$) in all exposure groups,with the highest concentration observed in group 5. Methemoglobin was significantly increased ($p < .01$) in group 5. Deoxyhemoglobin was decreased in groups 2 and 3, and significantly decreased ($p < .01$) in groups 4 and 5. Oxyhemoglobin was depressed in all exposure groups.

Glucose levels increased in groups 2, 3, and 4, and was significantly increased ($p < .01$) in group 5, as concentration and time increased (Figure 6). Serum electrolytes were within their respective biological ranges. The remaining serum chemistry parameters were within normal ranges.

Wet/Dry Lung Weight Determinations

There was no significant difference between control and exposure groups for percentage water in the lung, grams water in the lung per

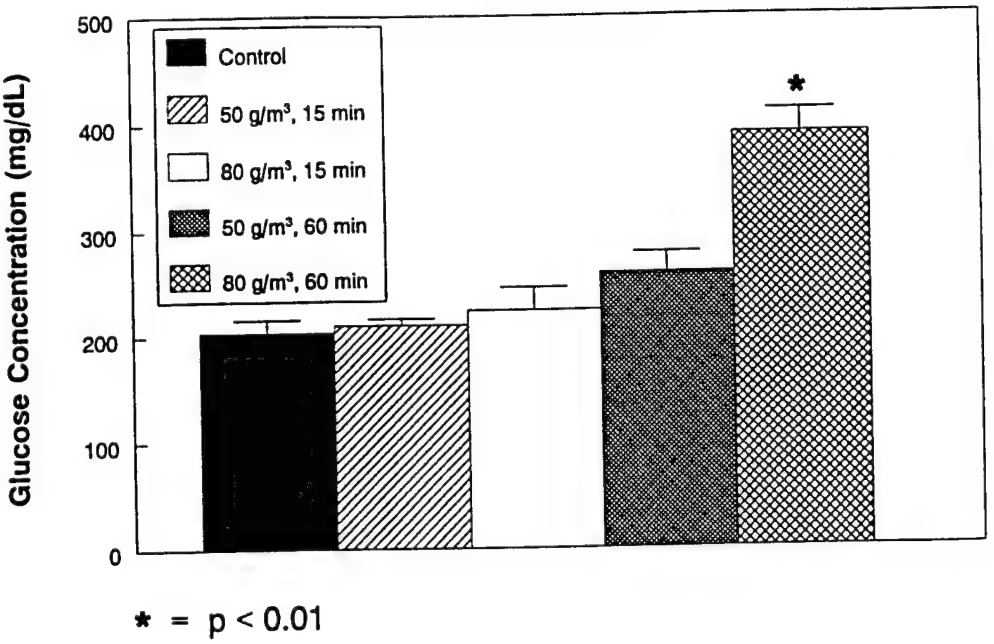

FIGURE 6. Glucose concentrations for Fischer 344 male rats exposed to the aerosol products of SFE Formulation A at load concentrations of 50 or 80 g/m³ for 15 or 60 min. Asterisk indicates significantly different than control at $p < .01$. Group 1 = control, group 2 = 50 g/m³ for 15 min, group 3 = 80 g/m³ for 15 min, group 4 = 50 g/m³ for 60 min, group 5 = 80 g/m³ for 60 min.

kilogram body weight, and grams solid (dehydrated) lung per kilogram body weight.

DISCUSSION

SFE Formulation A is considered a complete fuel, generating oxygen during the pyrolyzation process. Pellet ignition subsequently caused an increase in pressure within the exposure system. Operating the system in a dynamic state allowed the pressure pulse to be vented and also helped to entrain the aerosol into the chamber. Dissipation of the pressure pulse was observed just prior to the aerosol reaching its maximal concentration within the chamber. The dissipation occurred approximately 20 s postignition. Therefore, a 25-s delay was set before transforming the chamber from a dynamic state to a static state. This set delay provided a convenient and reproducible means of trapping the aerosol within the chamber. Oxygen generated during pyrolyzation creates a very high temperature at the ignition source. During the pyrolyzation of SFE, the temperature within the ignition chamber of the generator reached temperatures as high as 1200°C. However, there was no observed elevation of temperature within the exposure chamber.

The aerosol mass concentration within the chamber underwent an exponential decay throughout the exposure period (Figure 1). The decay constants were on the order of minutes. Two effects, agglomeration and gravitational settling, can be attributed to the decay in the concentration of an aerosol (Hinds, 1982). These effects were pronounced due to the static operating conditions within the chamber. In the case of the SFE, the aerosol remained of a respirable size throughout the exposure period even though the MMAD of the aerosol increased over time (Figure 2). This suggests that growth due to agglomeration outpaced the effects of gravitational settling. In a fire setting, however, SFE mass aerosol concentrations may remain elevated for longer periods due to the dynamic nature of the fire (i.e., the presence of thermal currents). The modal behavior of the exposure atmosphere within the chamber was similar to that observed in field studies (Kimmel et al., 1996).

The immediate physiological response to inhaling the resulting aerosol from pyrolyzed SFE was the primary focus of this study. During the 1-h exposure period, clinical observations of dyspnea, lack of coordination, and lethargy were noted. These clinical responses in the context of pyrolyzation of SFE suggested the formation of carbon monoxide during the pyrolyzation process. A 40% increase in carboxyhemoglobin levels for animals exposed to an SFE load of 80 g/m^3 for 60 min was also observed. This further supported the premise that carbon monoxide was a resultant product of SFE pyrolyzation. Carbon monoxide is often the product of incomplete combustion of carbon based material (World Health Organization, 1979). The only source of carbon in SFE is an epoxy resin (binder) that is used to shape the parent material into a pellet. Further investigation of the exposure environment after pyrolyzation confirmed the presence of carbon monoxide.

The available amount of hemoglobin is crucial in maintaining adequate gas exchange. Therefore, an increase in carboxyhemoglobin levels, such as seen in this study, will deplete the amount of hemoglobin available to transport oxygen and buffer blood pH (Lehninger, 1975). Reducing the ability to transport oxygen and buffer blood pH will impair gas exchange, producing a mild respiratory acidosis. During respiratory acidosis, serum glucose is elevated. This was observed in the study in a dose-dependent manner for all exposure groups. The observed apneustic breathing suggested an attempt to expel a noxious substance. Despite the clinical observations, no deaths occurred, and all animals recovered once placed in fresh air, suggesting concomitant conditions of mild carbon monoxide intoxication.

Edemagenesis was a primary concern because of the high concentration of KCl particles. The deposition and dissolution of large quantities of water-soluble salts, such as KCl, can result in the disruption of the alveolar/interstitial osmotic gradient. This gradient is a dynamic

condition responsible for maintaining extravascular fluid balance and preventing alveolar flooding (Staub, 1974). Therefore, inhalation of the SFE aerosol presented a distinct possibility of producing a pulmonary edematous response. For this study, the wet/dry lung weight ratio was used as a crude evaluation to determine if an edemagenetic response was elicited under the exposure conditions. There was no significant increase in wet/dry weight ratio for animals exposed to SFE. Therefore, frank pulmonary edema was ruled out as a factor in the immediate response to SFE aerosol inhalation. The lack of an elevated serum K^+ and/or Cl^- level may be attributed to any one of several factors, such as delayed dissolution within the lung, retention within the lung cells, or excretion by the kidneys. All are plausible explanations that need further investigation.

The results of this study indicate that inhaling the resulting aerosol from pyrolyzed SFE does not produce an immediate pulmonary edematous response. Therefore, it should not impair an individual's escape from a fire situation in which SFE has been released. These results, however, do not address possible lung injury or tissue damage that might lead to progressive lung disease. Even though pulmonary edema was not observed during this study, it is still an anticipated observation in future inhalation studies.

CONCLUSION

An acute toxicity was observed after rats were exposed to the resulting aerosol of pyrolyzed SFE Formulation A. Clinical observations were similar to those of carbon monoxide intoxication. An increase in carboxyhemoglobin was noted after each exposure to the resulting SFE aerosol. Carbon monoxide was produced during the pyrolyzation process, probably due to the incomplete combustion of the binding agent used in the parent material. The pyrolyzation process of SFE Formulation A also produced high concentrations of KCl particles. Impactor analysis indicated that the particles had an MMAD of 2–3 μm, an average concentration of 6.6–8.5 g/m^3, and a multimodal size distribution. These respirable aerosols appeared to be a pulmonary irritant, but did not cause immediate severe pulmonary edema. No immediate lethality was observed. No ocular or dermal irritation or histopathological lesions were detected. No evidence was observed of KCl being absorbed into the general circulation within 1 h postexposure. The concentrations tested in this study were in the range potentially useful for fighting fires, and the exposure time chosen was several times that estimated for the worst case scenario of delayed exit from a ship engine room. The high immediate survivability suggests that these materials should continue to be developed as fire-fighting agents. Further studies are planned as the fire-fighting agents continue to

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develop. The main emphasis of future studies will be to evaluate the physiological effect that atmospheres of high aerosol salt concentrations have on the lung and respiration.

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PERFORMANCE, FLUID MECHANICS, AND DESIGN OF A SMALL-ANIMAL, WHOLE-BODY INHALATION EXPOSURE CHAMBER

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A 0.7-m^3 inhalation exposure chamber was designed for continuous whole-body exposure of up to 64 small laboratory rodents to a variety of airborne toxicants using either single- or multiple-tier configuration. Though similar in design to conventional exposure chambers currently in use, several design modifications were incorporated to improve chamber performance with regard to both animal maintenance and toxicant distribution within the chamber. Several approaches were taken to assess chamber performance characteristics. Repeated, random-order serial sampling of 27 discrete loci within the exposure volume was used to determine both spatial and temporal distribution of polydisperse NaCl aerosols (1.3 μm MMAD, 1.65 σ_g). The collective spatial and temporal deviation of aerosol concentration within the exposure volume as a whole ranged from 3.5 to 5.2% as a function of single versus multiple-tier configuration. Application of a mathematical model of mixing characteristics in dynamic-flow reaction vessels demonstrated that the effective mixing volume in the chamber varied from 50 to 65% of total chamber volume, depending upon configuration. Computational fluid mechanics methods used to model flow structure within the exposure volume demonstrated that buoyant forces dominated flow structure development. Flow structure was shown to be sensitive to small changes in temperature. There was a marked agreement between assessment of chamber performance by flow structure analysis and assessment of performance by more conventional methods.

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Uniform distribution of the test material throughout an inhalation exposure apparatus is desirable to minimize nonbiologically caused variation of the inhaled dose. However, this often does not prevail; therefore, it is important to characterize distribution of test material within an exposure chamber to determine the impact of nonhomogeneities of exposure, if any, on animal response to the test material. Most importantly, knowledge of the chamber distribution characteristics is necessary to evaluate how representative selected sampling points for routine measurements are of the entire exposure volume. In fact, demonstration of chamber homogeneity and representative sampling is sometimes used as one of the criteria for proof of capability when contracting inhalation toxicity studies. Several approaches have been taken toward analyzing exposure chamber performance. These include (1) flow visualization techniques (Moss et al., 1982) and (2) quantitative determinations of the rate of dispersion and the distribution of vapors and aerosols (Hemenway et al., 1982; Yeh et al., 1986). Several methods were chosen to analyze the performance of an exposure chamber designed for use at the Tri-Services Toxicology Consortium at Wright-Patterson Air Force Base, Ohio.

Exposure chamber design based on a rudimentary understanding of chamber flow patterns has been a useful approximation for actual chamber operation. Indeed, flow visualization models have been used to finalize chamber design. However, chamber operation remains an approximation to design. Recent advances in computational fluid dynamics offer a closer simulation to actual air flow (at significant computational cost). Nevertheless, we applied computational fluid dynamic simulation modeling to analysis of an actual inhalation chamber. The purpose of this investigation was twofold: (1) to examine a modification to 27-in chamber inlet geometry while using various chamber characterization methods; (2) to examine thermal effects on chamber performance. Thus, this investigation examines the major factors influencing performance of these types of chambers in general.

A "snapshot" of test material distribution in the chamber was obtained by analysis of the overall and local variations of aerosol concentration measured at several discrete locations within the chamber. Concentration and differences in the magnitude of local variation of concentration were analyzed to determine the combined temporal and spatial variation within the chamber and to identify localized abnormalities of aerosol distribution and variability. To facilitate characterization of this aspect of chamber performance an attempt was made to isolate various contributing factors affecting test material within the exposure chamber. Sequential measurements were made at a central reference point to isolate the temporal component of combined spatial and temporal variation (Carpenter et al., 1987). Chamber integrity (leakage) was determined by a modification of the method of Mokler

and White (1983). Chamber mixing characteristics were determined using the method of Cholette and Cloutier (1959). Numerical solutions of laminar Navier-Stokes equations (Yerkes & Faghri, 1991, 1992a, 1992b) were used to model thermal effects on chamber flow structure. These solutions were verified in both a chamber mock-up and the full-size chamber, and attributes of flow structure were correlated with variation in test material distribution.

METHODS AND MATERIALS

Design and Operation

The chamber (referred to as the THRU chamber, for Toxic Hazards Research Unit) is a slightly larger (690 L), modified version of the 27-in vertical flow exposure chamber originally described by Hinners and associates (1968) (Figure 1). Exposure capacity was either 64 mice or 32 rats. The chamber is exhausted through a distributive manifold similar to that described by Carpenter and Beethe (1981) and has an annular-orifice, continuous opposed jet. This inlet geometry was designed so that inlet cross-sectional area could be adjusted to manipulate inlet velocity for the purpose of enhancing test material mixing and to minimize rotational and reflux flow. Chamber design allowed

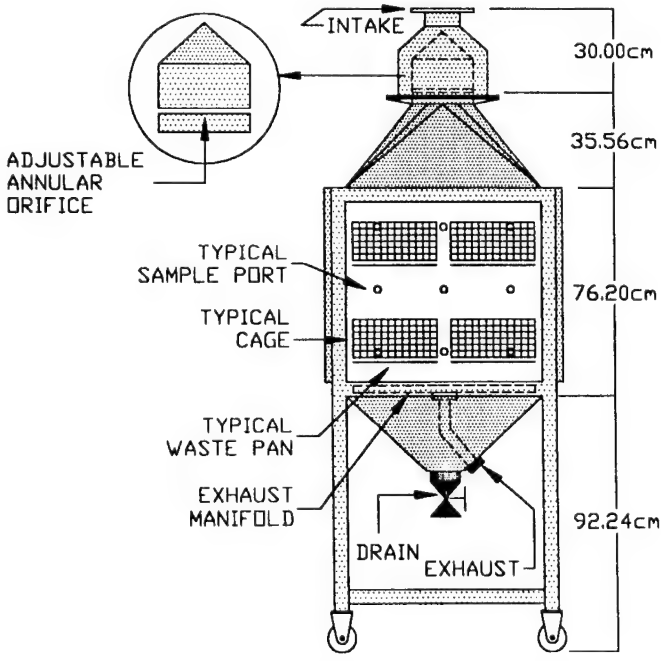


FIGURE 1. Schematic of the 0.7-m^3 THRU whole-body inhalation exposure chamber.

the use of heat-tempered plate glass for two side walls and the chamber door. The rear wall of the chamber and the plenums were fabricated out of stainless steel plate. For this investigation, the chamber was operated in three different configurations, described in Table 1. For normal operation, two tiers of four (two per tier) horizontal cage units with excreta catch pans were suspended axisymmetrically about the vertical axis of the exposure volume. A 10-cm gap was left between the cage units themselves and between cage units and chamber walls on each tier. The chamber was operated at a flow rate of 162.2–170.2 L/min at 1.03–1.57 mm Hg subambient pressure maintaining 14.1–14.8 chamber volume changes per hour.

Test Material Generation and Sampling

Polydisperse NaCl aerosols were generated using a modified, large-reservoir Retec compressed-air nebulizer (model X-70/N, Cavitron Corp., Portland, OR). Prior to countercurrent injection into the chamber inlet air stream, the nebulizer output was passed through a conditioning vessel maintained at 98.8°C in which a 10-mCi ^{85}Kr deionizer was located (Figure 2). Mass concentration and aerosol size distribution data for the test atmospheres are given in Table 2. Nine sampling ports located in the rear wall were fitted with 0.64-cm-diameter stainless steel probes that could be positioned at various depths into the exposure volume. The center port also was fitted with reference probe fixed in the exact center of the exposure volume. The exposure volume was divided into 27 identical 12.37-L cuboidal cells (Figure 3), and the center of each cell served as the sampling loci. In a trial run, each locus was sampled in random order and 16 reference point samples were interleaved between loci samples so that at least every third sample taken was from the reference point. Five trials were made for each of the three chamber test configurations. Aerosol concentration and size distribution were measured with an aerodynamic particle sizer (APS model 33B with a 3302 100:1 diluter, TSI, Inc., St. Paul, MN). Each sample was taken at 1 L/min for 2 min. The time between samples was 0.5 min. Concentration at the chamber exhaust port was measured continuously with a RAM-S (MIE, Inc., Bedford, MA) nephlometer (Figure 4) for use in determining chamber mixing characteristics, through examination of changes in aerosol concentration on emptying of the chamber by the method of Cholette and Cloutier (1959).

TABLE 1. Chamber test configuration

Configuration 1	Empty
Configuration 2	Two tiers, four cages (two per tier) with excreta pans
Configuration 3	Same as config. 2 with full compliment of rats (32)

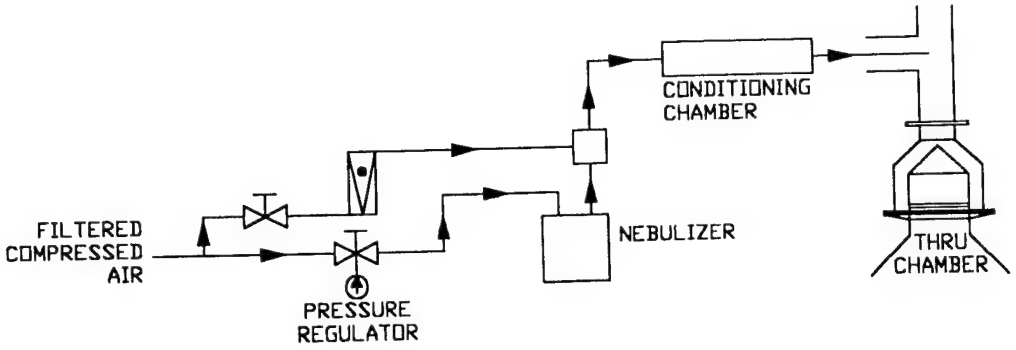


FIGURE 2. Schematic of the aerosol generation system.

Spatial and Temporal Variation

Spatial and temporal variation of concentration within and between cells was determined as described by Carpenter and colleagues (1987). Briefly, the coefficient of variation of concentration and variability at the fixed reference sampling point were considered representative of the temporal component of combined variation. Spatial variation was calculated by subtraction of this from the overall observed variation within a given cell.

Numerical Simulation of Flow Structure

Numerical solutions of the two-dimensional, incompressible, time-dependent Navier–Stokes equations were used to develop models of flow structure in the chamber. These time-dependent equations were used to solve for either a steady-state or stead-periodic solution. The Boussinesq approximation was used to account for buoyancy effects in the governing of the equations given next (Yerkes & Faghri, 1991, 1992a, 1992b).

Conservation of mass:

$$\frac{\partial v}{\partial y} + \frac{\partial w}{\partial z} = 0$$

TABLE 2. Chamber concentration and aerosol size distribution

	Concentration (mg/m^3)	MMAD (μm)	Geometric standard deviation (σg)
Config. 1	5.76 ± 0.354	1.39 ± 0.037	1.66 ± 0.025
Config. 2	5.78 ± 0.110	1.28 ± 0.018	1.61 ± 0.011
Config. 3	4.37 ± 0.241	1.35 ± 0.023	1.61 ± 1.012

Note. All values are $\bar{X}$ ± SD. Data are grand $\bar{X}$ for five trials of n = 27 each. MMAD, mass median aerodynamic diameter.

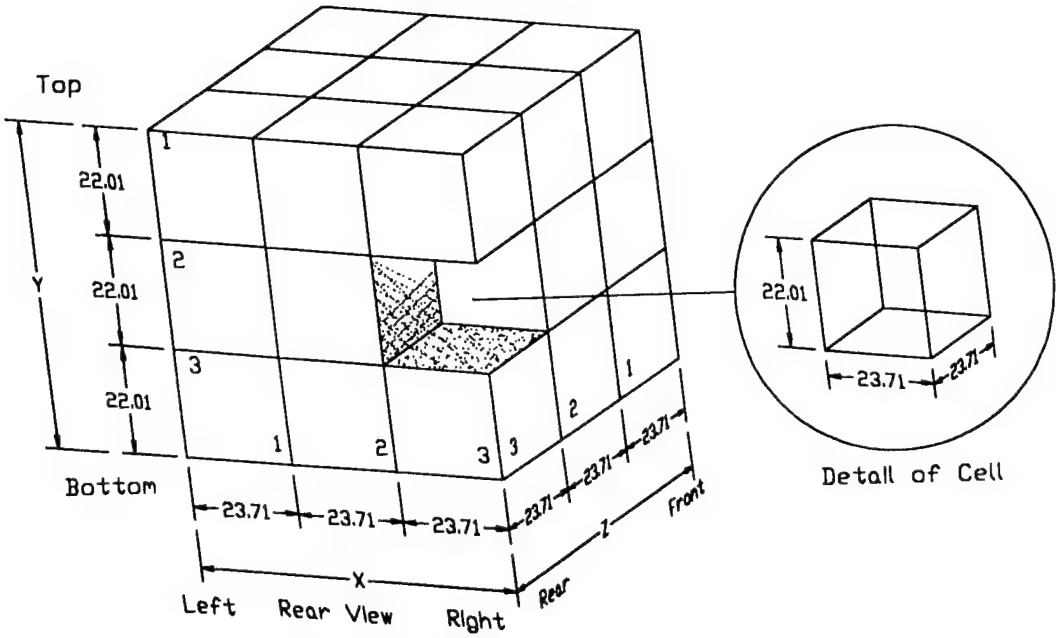


FIGURE 3. Diagram of sampling cells D(27) within the chamber effective volume. All dimensions in cm; numbers are cell coordinate designations.

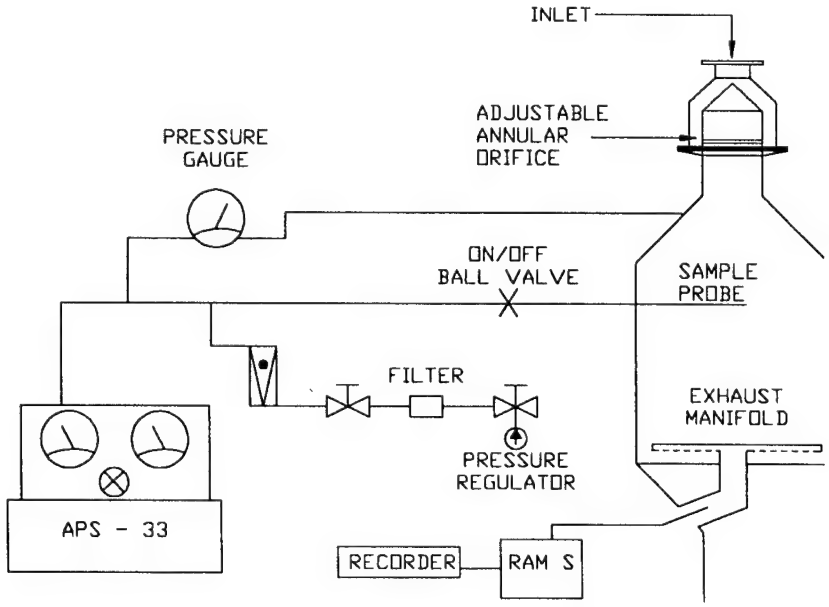


FIGURE 4. Schematic of the aerosol sampling system.

Conservation of momentum:

$$\rho\left(\frac{\partial v}{\partial t} + v\frac{\partial v}{\partial y} + w\frac{\partial v}{\partial z}\right) = -\frac{\partial p}{\partial y} + \mu\left(\frac{\partial^2 v}{\partial y^2} + \frac{\partial^2 v}{\partial z^2}\right)$$

$$\rho\left(\frac{\partial w}{\partial t} + v\frac{\partial w}{\partial y} + w\frac{\partial w}{\partial z}\right) = -\frac{\partial p}{\partial z} + \mu\left(\frac{\partial^2 w}{\partial y^2} + \frac{\partial^2 w}{\partial z^2}\right) + \rho g\beta(T - T_{in})$$

Conservation of energy:

$$\rho\left(\frac{\partial T}{\partial t} + v\frac{\partial T}{\partial y} + w\frac{\partial T}{\partial z}\right) = \frac{k}{C_p}\left(\frac{\partial^2 T}{\partial y^2} + \frac{\partial^2 T}{\partial z^2}\right)$$

Boundary conditions:

$$\text{Inlet: } t \geq 0,\ v = 0,\ w = w_{in} = 4.1 \times 10^{-3} \text{ m/s},\ T = T_{in} = 300°\text{K}$$
$$\text{Outlet: } t \geq 0,\ v = 0,\ p = 0$$
$$\text{Vertical end wall: } t \geq 0,\ v = 0,\ w = 0,\ T = T_w = 297°\text{K}$$
$$\text{Baffle: } t \geq 0,\ v = 0,\ w = 0$$
$$\text{Heat sources: } t \geq 0,\ T = 305°\text{K}$$

where y is the horizontal coordinate; z the vertical coordinate; v the y-direction velocity (m/s); w the z-direction velocity (m/s); μ the dynamic viscosity (kg/m/s); C_p the specific heat (J/kg/K); ρ the fluid density (kg/m^3); t the time (s); β the coefficient of thermal expansion, $-(1/\rho)\ (\partial\rho/\partial T)$ (K^{-1}); k the thermal conductivity (W/m/K); T the temperature (K); T_{in} the inlet temperature (K); T_w the end vertical wall temperature; h the hydrostatic height (m); W_{in} the vertical velocity at chamber inlet; g the acceleration due to gravity (m/s); and p the pressure due to motion and hydrostatic pressure.

Characteristic flow structures were modeled for three generalized conditions of either negative, positive, or zero differences between the inlet and vertical wall temperatures. Flow structure simulations were derived for operation with and without simulated animal heat sources. Detailed descriptions of simulation parameters, numerical schema, and assumptions have been reported previously (Yerkes & Faghri, 1991, 1992a, 1992b). The solution grid was 100 × 100 imposed on the vertical plane corresponding to the front of the chamber effective volume, the upper boundary of which was the base of the pyramidal inlet plenum and the lower boundary of which was the horizontal plane corresponding to the exhaust manifold (i.e., the base of the inverted pyramidal exhaust plenum). The chamber inlet cone was assumed to provide uniform velocity distribution resulting from a constant exhaust

pressure across the exhaust plane. Experimentally the velocity and thermal simulations were verified using a Plexiglas–aluminum scaled model of the exposure chamber in which a temperature gradient was produced and maintained by a wall-mounted water jacket (Yerkes & Faghri, 1991, 1992a, 1992b). Temperature measurements were made with iron-constantin thermocouples calibrated to within ±0.1°C using a NIST traceable thermometer (±0.05°C, Ever Ready Thermometer Co., New York, NY). Hot-wire anemometery (model 1211-10, TSI, Inc., St. Paul, MN) was used to verify velocity simulations. Flask heating mantles were used in the chamber mockup to simulate animal heat sources. The mantles were maintained at 32°C to simulate heat output of a 250- to 300-g rat (Bernstein & Drew, 1980; Mauderly, 1986).

Statistics

Comparisons of intercellular differences in concentration and variability were made using either Tukey's studentized range method or Ryan-Einot, Gabriel, Welsh factorial analysis of variance, where appropriate.

RESULTS

Chamber Leak Rate

The initial calculated leak rate was 1.28×10^{-2} L/min, corresponding to 1.86×10^{-5}% of the chamber volume per minute and 7.7×10^{-5}% of the average chamber operating flow (166.4 L/min). Prior to separate trial runs, chamber integrity was tested (Mokler & White, 1983), and remedial maintenance on chamber gaskets and seals was performed to maintain this or a lower leak rate in order to minimize the effects of leakage on other measurements of chamber performance.

Mixing Characteristics

Aerosol concentration decay in the chamber from steady state was determined by plotting concentration at the exhaust semilogarithmically as a function of time post generator shutdown for each of the chamber configurations (Figure 5). The data were fitted to the general exponential decay function:

$$C/C_o = e^{-Qt/V}$$

where C is the concentration at time t, C_o the steady-state concentration, Q the chamber flow rate, and V the chamber volume. From these plots the fractions of chamber volume were calculated (Table 3), which were either dead space volume, volume well mixed with incoming air, or incoming air shunted to the chamber exhaust as pis-

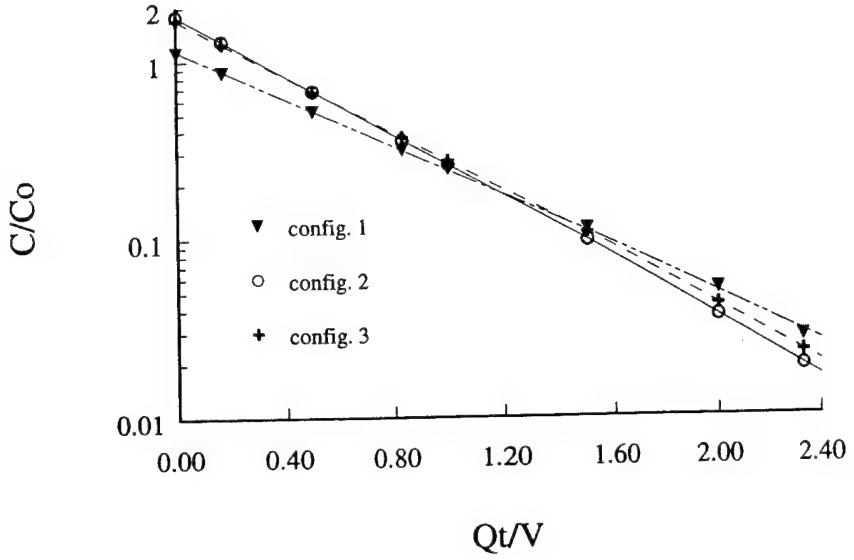


FIGURE 5. Decay of chamber concentration:

Config. 1, $C/C_o = -0.002 + 1.13e^{-1.54(Qt/V)}$, $r^2 = 0.99999$
Config. 2, $C/C_o = -0.0002 + 1.80e^{-1.95(Qt/V)}$, $r^2 = 1.0000$
Config. 3, $C/C_o = -0.002 + 1.71e^{-1.87(Qt/V)}$, $r^2 = 0.99997$

Data are mean of five trials; C is the concentration at time t, C_o the steady-state concentration, Q the chamber flow, and V the chamber volume.

ton (plug) flow (Carpenter et al., 1987). The three distinct "volume" types are characterized by their predominant mass, heat, and fluid transport mechanisms. In dead space diffusion is the primary transport mechanism. Transport in well-mixed (mixed convection) volume is due to diffusion combined with advection due to fluid velocity. Transport in plug flow (forced convection) is primarily due to advection due to fluid velocity. The volume of the lower plenum (11.9% total volume) was actually located below the chamber exhaust plane (produced by the exhaust manifold). Using the present experimental procedure of plotting concentration decay as opposed to monitoring of a pulsed test material input, evacuation of this lower plenum volume analytically appeared as piston or short-circuit flow when in effect it could be considered as structural dead space (vs. fluid dynamic dead space). Fractional chamber volumes adjusted for this structural dead space are shown in parentheses in Table 3.

Spatial and Temporal Variation

Combined spatial and temporal variation of average chamber concentration ranged from 3.5 to 5.2% depending on chamber configuration. On average, spatial variation accounted for 85% of the total.

TABLE 3. Chamber mixing characteristics

	Plug flow (short circuit–forced convection) volume (%)	Well-mixed (mixed convection) volume (%)	Dead space (diffusion) volume (%)
Config. 1	11.7[a] (0)	64.7	23.6 (35.3)
Config. 2	27.2 (15.5)	51.3	21.5 (36.9)
Config. 3	29 (17.3)	53.9	17.1 (28.3)
Mean[b]			
Config. 1 and 2	14.1	58.0	36.1
Simulation 1[c]	13.5	64.4	25.1
Simulation 2[d]	11.7	49.2	39.4
Mean simulation	12.6	56.8	32.3

Note. All values are percent of the total chamber volume. Values in parentheses are adjusted for structural dead space volume percent.

[a]Corresponds to approximately 11.9%, which is the volume percent of the lower plenum located below the exhaust manifold, which analytically behaves as piston flow but is structural dead space.

[b]Values are adjusted for structural dead space—configuration 3 not considered, as simulations with internal heat sources were not considered.

[c]Temperature difference 1°C.

[d]Temperature difference 3.3°C.

Relative spatial versus temporal contributions to total variation, for all configurations, are shown in Table 4. No intercellular differences in concentration were found in the empty chamber (configuration 1). In configuration 2 (Figure 6), significant differences in concentration between individual cells were found. Concentration in a block of three cells at the top left side (viewed from the rear) of the chamber was significantly higher than in all other cells. Concentration in four cells located just below the upper tier of pans (middle layer of the chamber) was significantly lower than in all other cells. Concentration in a single cell in the bottom layer of the exposure volume was significantly lower than in all other cells. With animals in the chamber

TABLE 4. Chamber spatial and temporal variation of concentration as coefficient of variation

	Total	Spatial	Temporal	Temporal/total[a]
Config. 1	3.54 ± 0.526	2.76 ± 0.529	0.78 ± 0.121	0.22
Config. 2	5.24 ± 0.551	4.52 ± 0.3533	0.71 ± 0.509	0.14
Config. 3	4.25 ± 1.029	3.82 ± 0.338	0.43 ± 0.111	0.1

Note. Data are grand $\bar{X}$ values for five trials, $n = 27$ each.

[a]Data are fractional portion temporal variation of total variation (temporal/total). All other values are $\bar{X} \pm SD$.

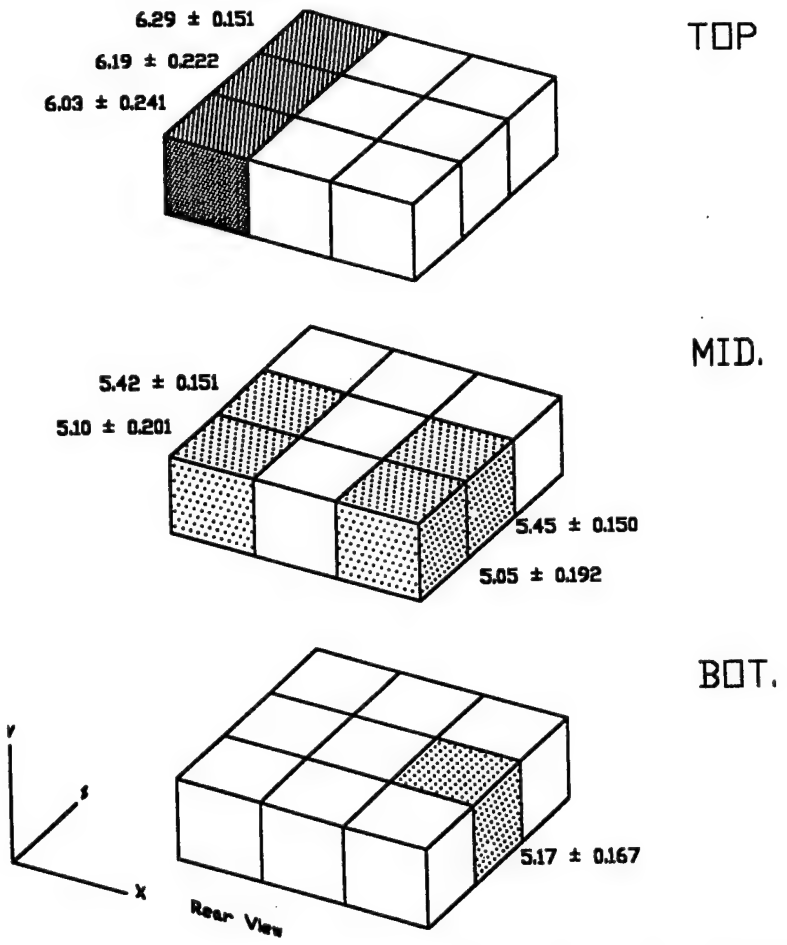


FIGURE 6. Chamber concentration profile, configuration 2. Data are mean of five trials. Dark shaded cells have significantly higher concentrations than all other cells, $p \leq .05$. Light shaded cells have significantly lower concentrations than all other cells, $p \leq .05$.

(configuration 3, Figure 7) no cellular differences in concentration were observed in the top or bottom layers of the exposure volume. However, as in configuration 2, the same four cells in the middle layer had concentrations significantly lower than all other cells. Differences of intercellular variability (relative magnitude of the variation of cellular concentration) were not observed in the empty chamber (configuration 1). In configuration 2 (Figure 8), two cells, located at the rear and to the sides of the middle layer, were significantly more variable than all other cells. One other cell in this layer was significantly more variable than all but 5 of the other 27 cells. The addition of animals to the chamber (configuration 3, Figure 9) changed

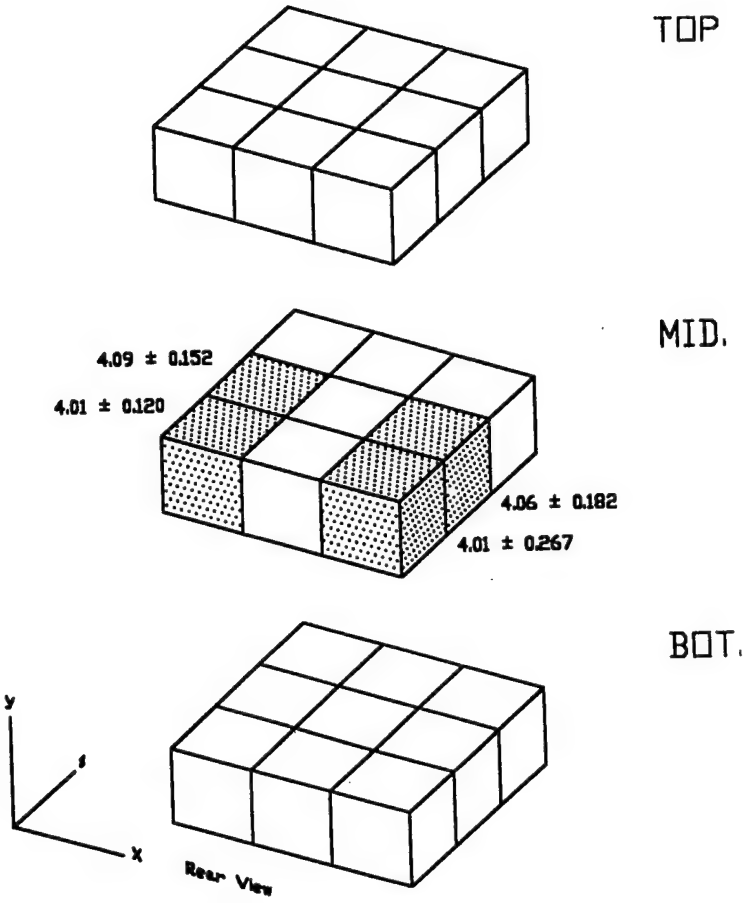


FIGURE 7. Chamber concentration profile, configuration 3. Data are mean of five trials. Shaded cells have significantly lower concentrations than all other cells, $p \leq .05$.

the pattern of variability in the chamber. All nine cells located next to the metal rear wall of the chamber were found to have significant differences of variability. All three cells in a block in the top layer of the exposure volume were significantly less variable than all other cells, whereas the corresponding blocks of three cells each in the middle and bottom layers of the exposure volume were significantly more variable than all other cells.

Numerical Simulation of Flow Structure

Numerical simulations of thermal effects on chamber flow structure were performed setting inlet and upper exposure volume temperature to vertical end-wall temperature gradients at 0, 1 (higher inlet), 1 (lower inlet), and 3.0°C (high inlet). In each case the end wall

(exhaust side) temperature was set at 24°C, which was within normal chamber operating temperature ranges. The first three gradient ranges are those commonly encountered most frequently during routine exposures. The latter differential mimicked extreme conditions where inlet temperatures may be high due a combination of factors including animal loading, environmental (room) temperature excursions, and inadequate dissipation of generator heat output from, for instance, heated vaporizers or aerosol dryers. A 3°C temperature gradient also could be imposed to use thermal effect on flow structure to influence test material distribution (vide infra). An inlet temperature of 27.3°C also represents an extreme condition, which, however, can be attained, periodically, in the course of chronic studies, particularly when many of the aforementioned factors are not under optimal control. The simu-

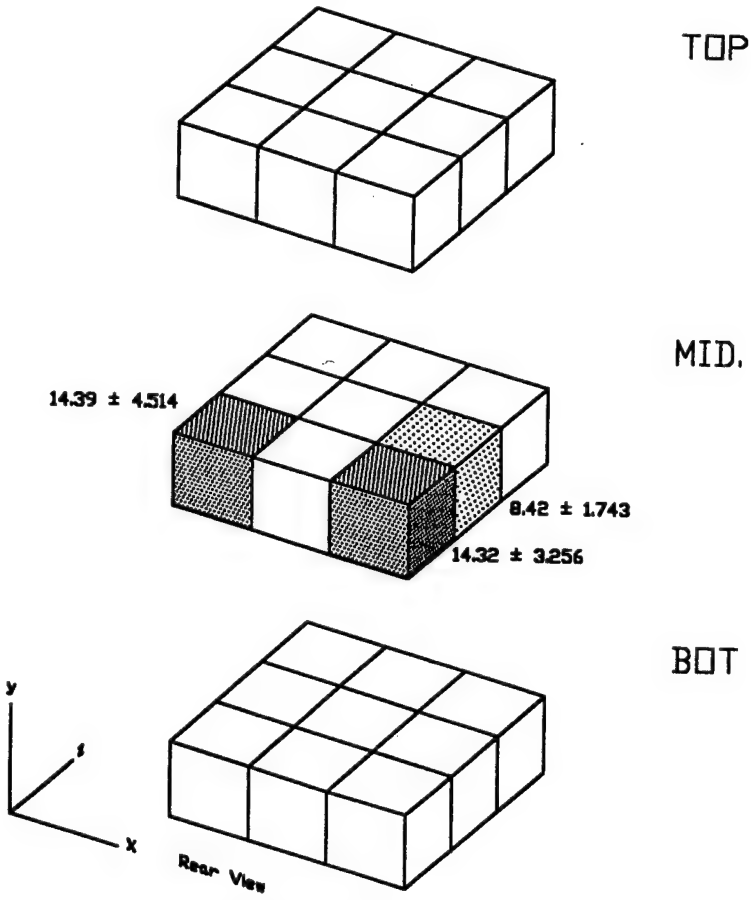


FIGURE 8. Chamber cellular variability profile, configuration 2. Data are mean of five trials. Dark shaded cells are significantly more variable than all other cells, $p \leq .05$. Light shaded cells are significantly more variable than remaining (nonshaded) cells, $p \leq .05$.

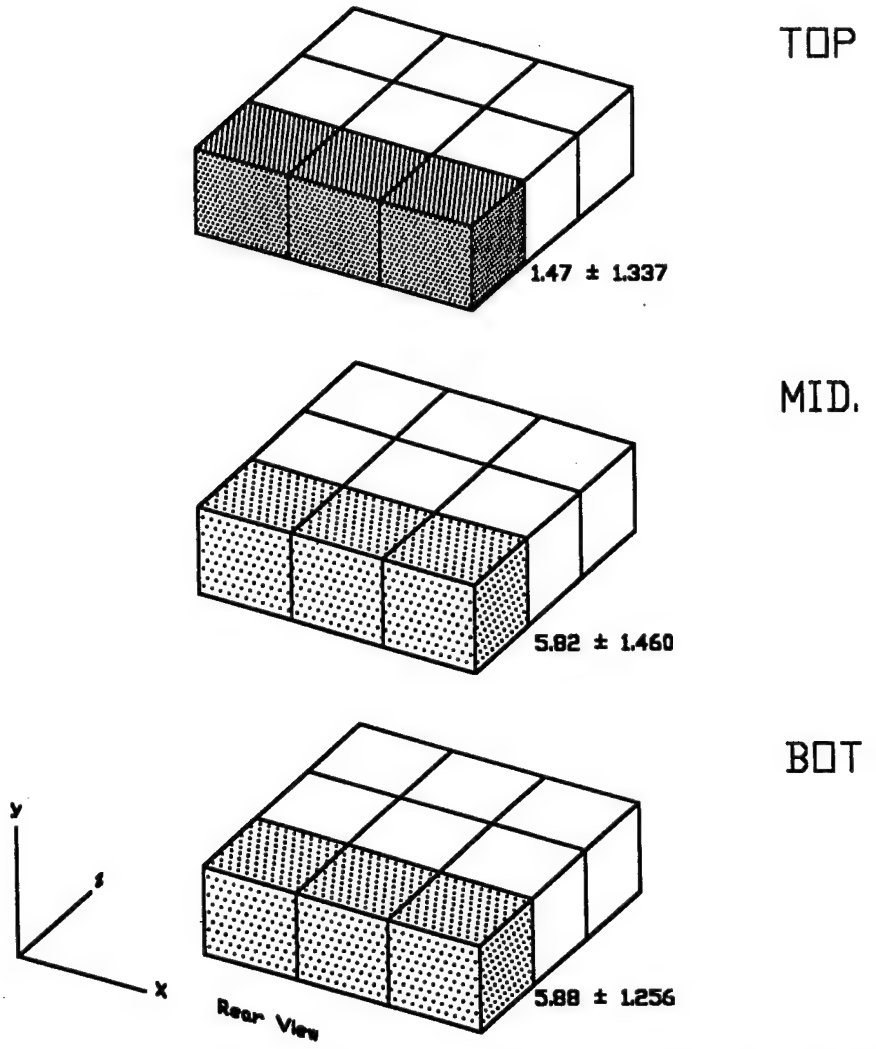


FIGURE 9. Chamber cellular variability profile, configuration 3. Data are mean of five trials. Dark shaded cells are significantly less variable than all other cells, $p \leq .05$. Light shaded cells are significantly more variable than all other cells, $p \leq .05$.

lations demonstrated that the development of flow structure within the chamber effective volume was characteristic and different for different inlet and vertical end-wall temperature gradients. Chamber flow and thermal structures were found to be similar. In a chamber without internal heat sources and zero temperature difference between the inlet and end walls (Figure 10) the upper baffles (excreta catch pans) diverted flow, resulting a steady-state flow structure consisting of a central core of higher velocity downward flow and increased velocity

downward flow along the vertical walls. The lower baffles deflected flow from the side walls horizontally across the upper surface of the lower baffles toward the chamber vertical centerline. In a chamber without internal heat sources and lower inlet than vertical end wall temperature (Figure 11) the flow structure consisted of a central core of downward flow with reflux upward from the exhaust plane eventually flowing along the walls toward the inlet. This reflux flow is attributed to "opposed" buoyant forces. This asymmetric flow pattern was periodic, with the leading edge of accelerated central core flow oscillating between sides of the chamber vertical centerline at 300-s intervals. The depth to which this accelerated central core flow "penetrated" also was periodic. The typical flow structure in a chamber with an inlet temperature greater than the vertical end-wall temperature and no internal heat sources is shown in Figures 12 and 13. It may be noted that an increasing ΔT results in a greater portion of the mass flow being directed toward the vertical walls, with an eventual elimination of the central core flow. With a high enough ΔT the baf-

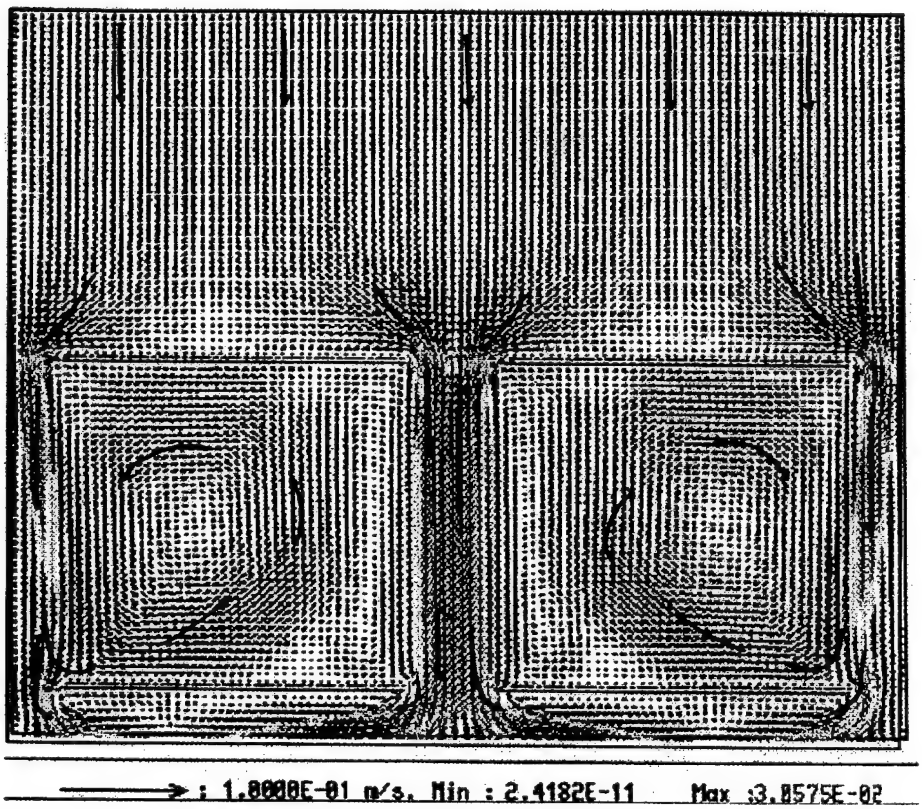


FIGURE 10. Chamber flow and thermal pattern without animals. Temperature difference = 0°C. Velocities: inlet = 4.1×10^{-3}, minimum = 2.42×10^{-11}, maximum = 3.05×10^{-1} m/s.

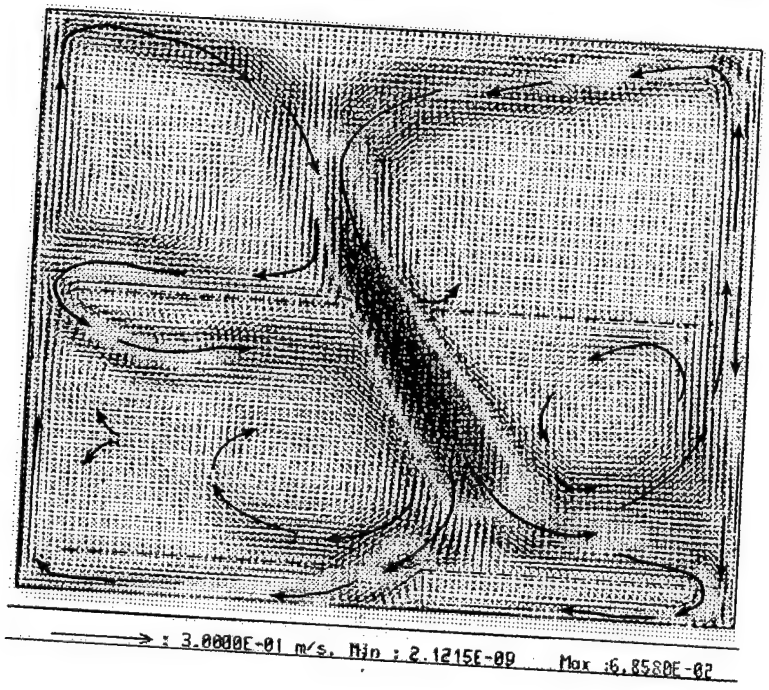


FIGURE 11. Chamber flow and thermal pattern without animals. Temperature difference = 1.0°C, inlet = 23.0°C, end wall = 24.0°C. Velocities: inlet = 4.1×10^{-3}, minimum = 2.12×10^{-9}, maximum = 6.86×10^{-2} m/s.

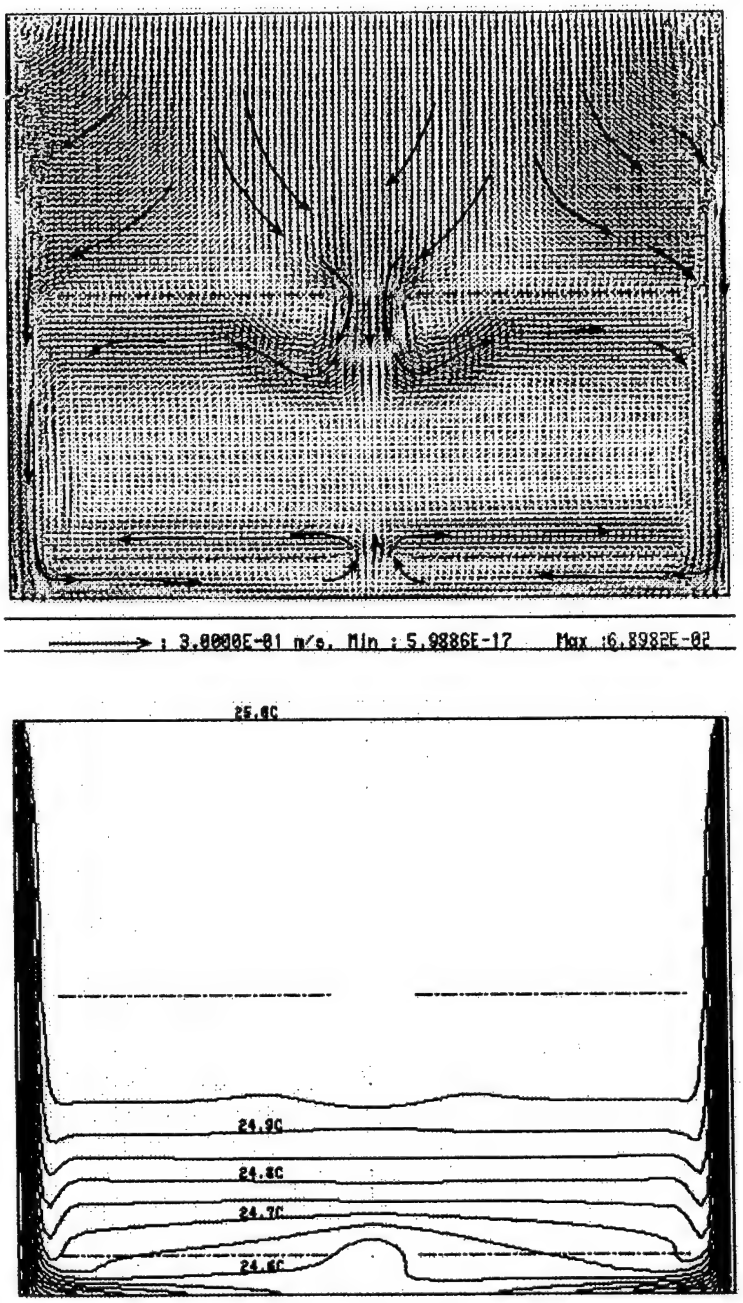


FIGURE 12. Chamber flow and thermal pattern without animals. Temperature difference = 1.0°C, inlet = 25.0°C, end wall = 24.0°C. Velocities: inlet = 4.1×10^{-3}, minimum = 5.19×10^{-17}, maximum = 6.90×10^{-2} m/s.

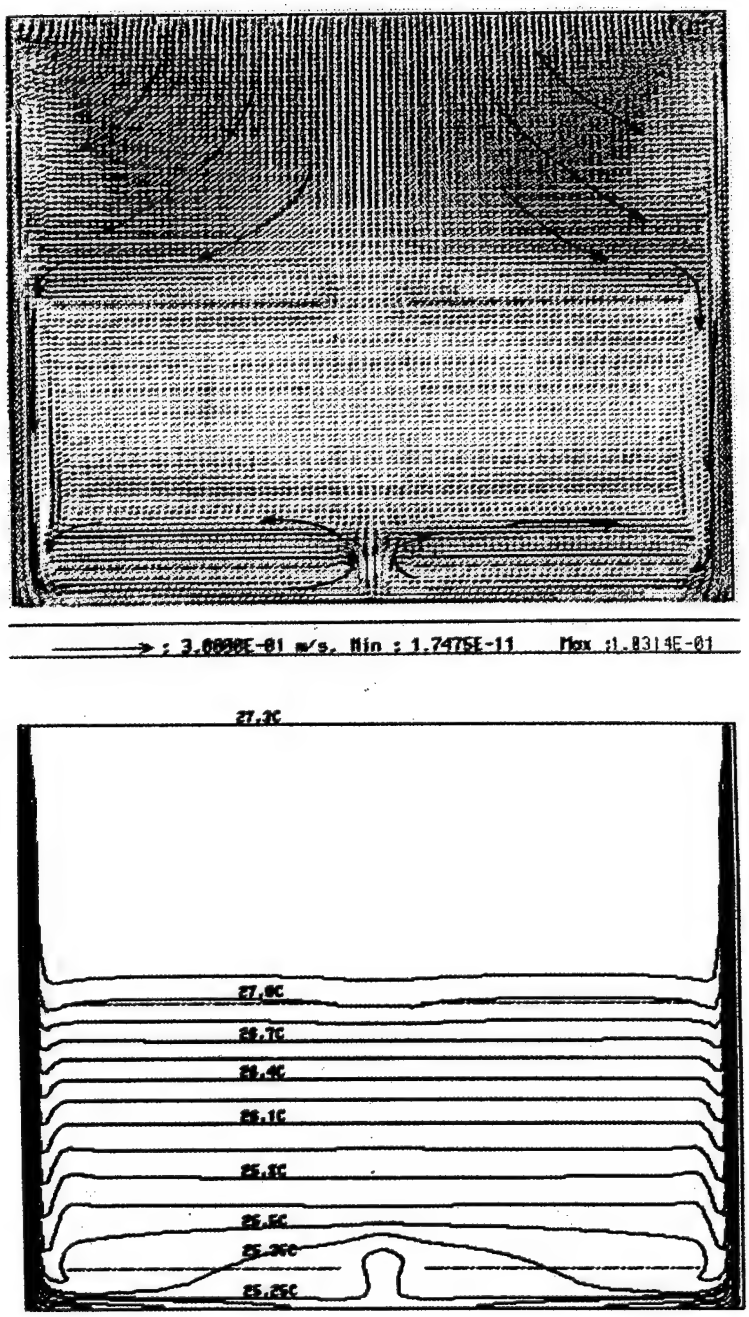
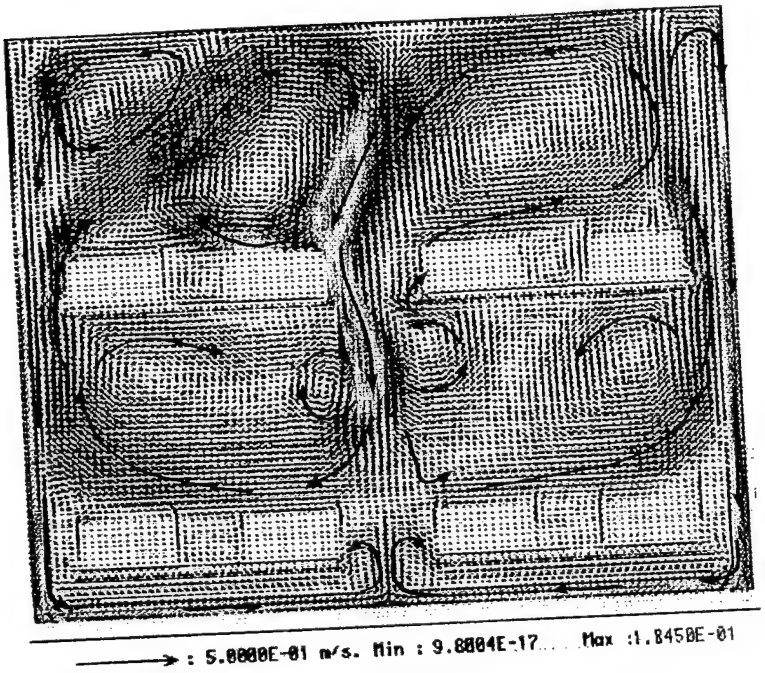


FIGURE 13. Chamber flow and thermal pattern without animals. Temperature difference = 3.3°C, inlet = 27.3°C, end wall = 24.0°C. Velocities: inlet = 4.1×10^{-3}, minimum = 1.75×10^{-11}, maximum = 1.03×10^{-1} m/s.

fles have a greatly reduced influence on flow. The upper baffles diverted flow toward the walls of the chamber, resulting in downward flow with increased velocity along the walls but without a distinct central core structure. There was horizontal reflux flow from the exhaust plane toward the chamber vertical centerline along the bottom of the lower baffles that turned 180 degrees at the centerline to become horizontal flow directed toward the walls across the upper surface of the lower baffles. This pattern also was steady-state, and three distinct types of flow were identified as either short-circuit, diffusion flow, and mixed convection flow. The area fraction of each flow type was determined for both the 1 and 3.0°C maximum temperature gradient conditions by analysis of vector plots. Dead space area fractions were defined as regions of low velocity where heat and mass transfer were dominated by diffusive properties. Well-mixed area fractions (mixed convection) were defined as regions where heat transfer and mass transfer were both diffused and convected due to moderate fluid velocities such as in a typical Bénard cell. Plug flow (forced convection) areas were defined as regions where heat transfer and mass transfer were primarily convected due to high fluid velocities such as in the central core and boundary layers along the chamber walls. A comparison was made between these area fractions and comparable chamber volume fractions as determined by the method of Cholette and Cloutier (1959; see Table 3).

The inclusion animal heat sources in the simulations resulted in marked changes in chamber thermal profile. With internal heat sources chamber flow structure also was shown to be predominantly buoyant in nature, marginally stable, oscillatory, and periodic. Figure 14 shows a series of flow patterns that represent approximately one half of a cycle of flow development (all patterns have a corresponding mirror image) occurring over a period of approximately 40 s. The most salient feature of these flow patterns was the formation, dissipation, and reformation of Bénard cells. As many as four Bénard cells formed above the upper set of baffles, and the formation, dissipation, and reformation of these cells resulted in a core of oscillatory flow between the upper baffles. High-velocity boundary-layer flow was maintained down the chamber walls, the thickness of which increased as the central core dissipated. There also was a characteristic opposing upward flow found next to this boundary-layer flow present throughout the flow structure cycle. Bénard cells also formed between the upper and lower sets of baffles, and the formation of these cells appeared to be influenced by the core flow. Core flow fluctuation, in turn, influenced the development of the Bénard cells. Reflux between the vertical walls and upper baffles also was influenced by the oscillating core flow. Reflux from the vertical end walls resulted in horizontal flow from the end walls toward the chamber vertical centerline below

(A)



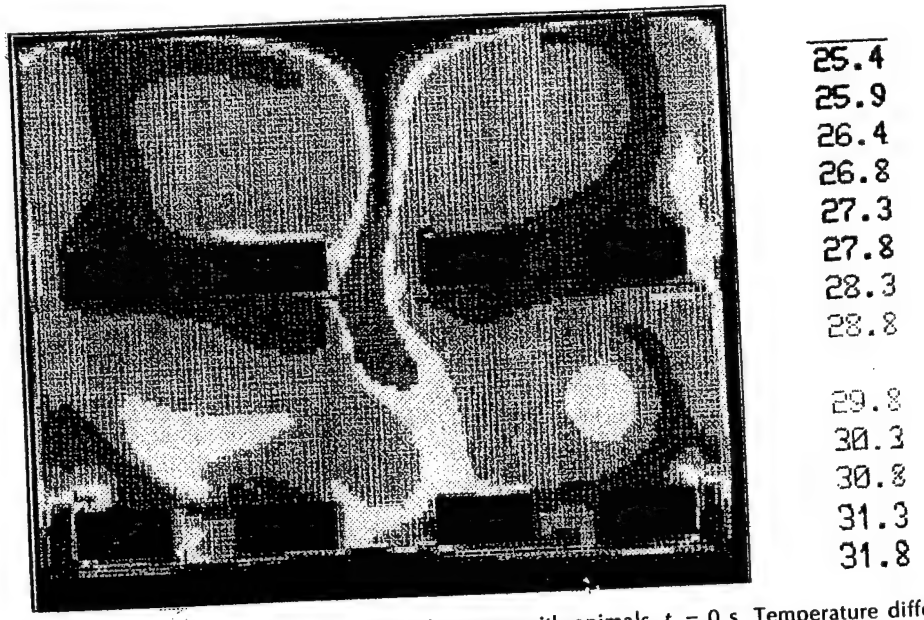
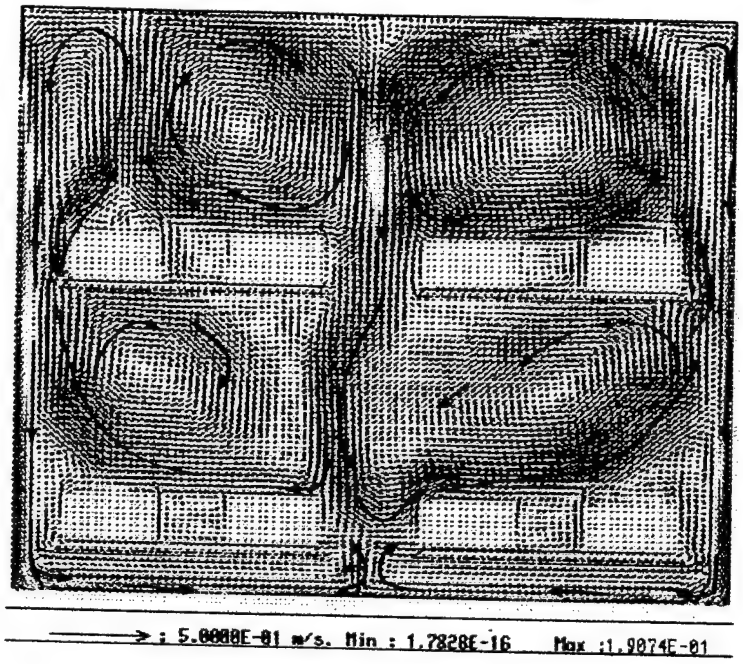


FIGURE 14. (a) Chamber flow and thermal pattern with animals, t = 0 s. Temperature difference = 3.0°C, inlet = 27.0°C, end wall = 24.0°C. Velocities: inlet = 4.1×10^{-3}, minimum = 9.80×10^{-17}, maximum = 1.85×10^{-1} m/s.



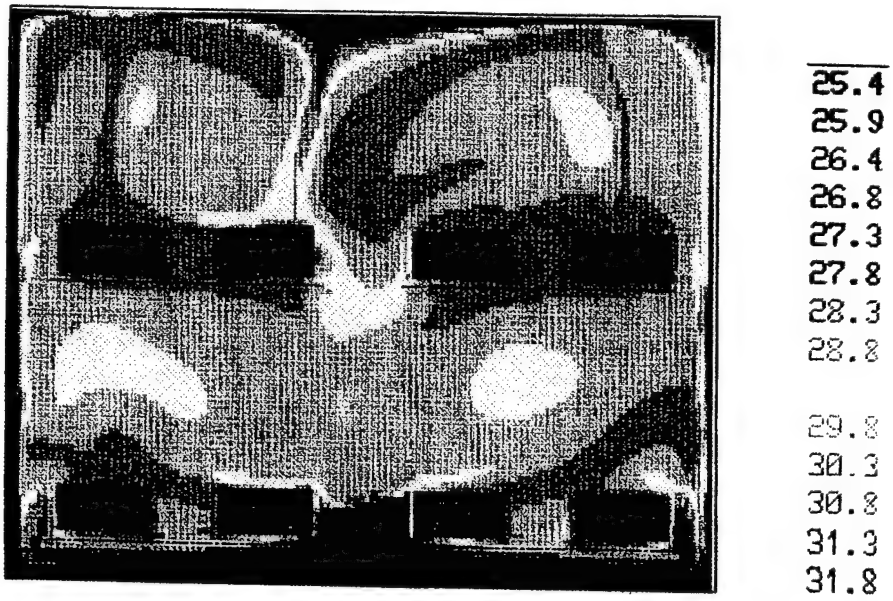
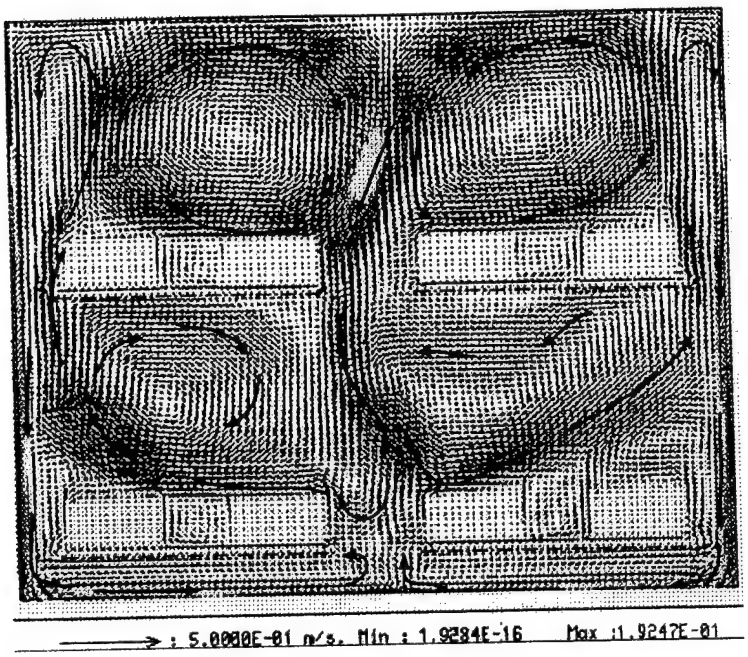


FIGURE 14. (*Continued*) (b) Chamber flow and thermal pattern with animals, t = +10 s. Temperature difference = 3.0°C, inlet = 27.0°C, end wall = 24.0°C. Velocities: inlet = 4.1×10^{-3}, minimum = 1.78×10^{-16}, maximum = 1.91×10^{-1} m/s.

(C)



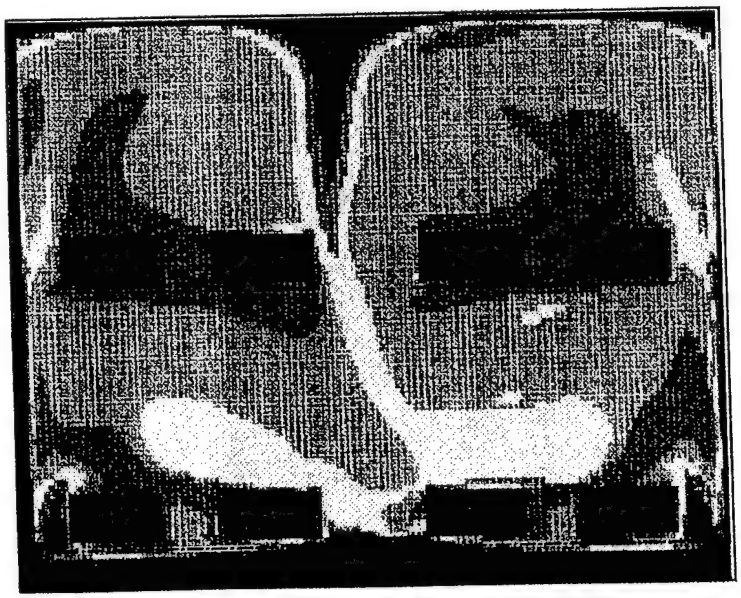


FIGURE 14. (*Continued*) (c) Chamber flow and thermal pattern with animals, t = +20 s. Temperature difference = 3.0°C, inlet = 27.0°C, end wall = 24.0°C. Velocities: inlet = 4.1×10^{-3}, minimum = 1.93×10^{-16}, maximum = 1.92×10^{-1} m/s.

(D)

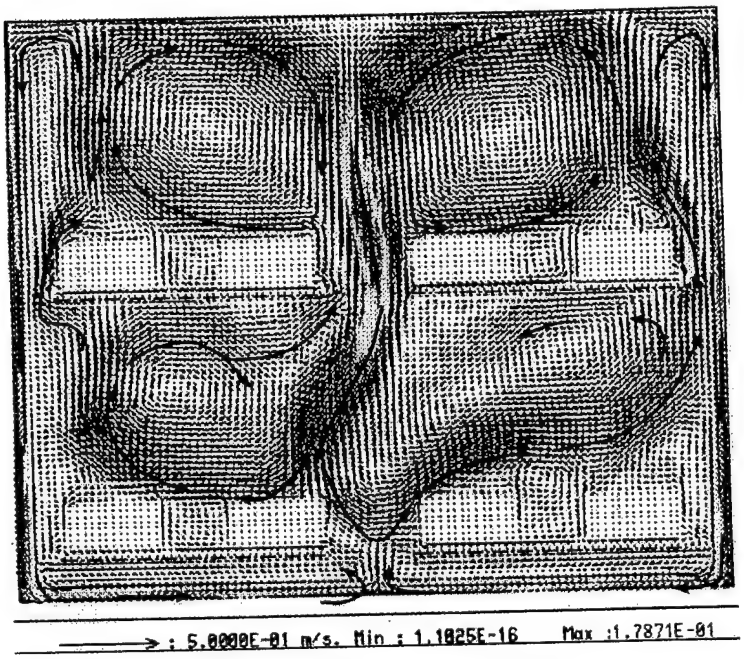


FIGURE 14. (*Continued*) (d) Chamber flow and thermal pattern with animals, t = +30 s. Temperature difference = 3.0°C, inlet = 27.0°C, end wall = 24.0°C. Velocities: inlet = 4.1×10^{-3}, minimum = 1.10×10^{-16}, maximum = 1.79×10^{-1} m/s.

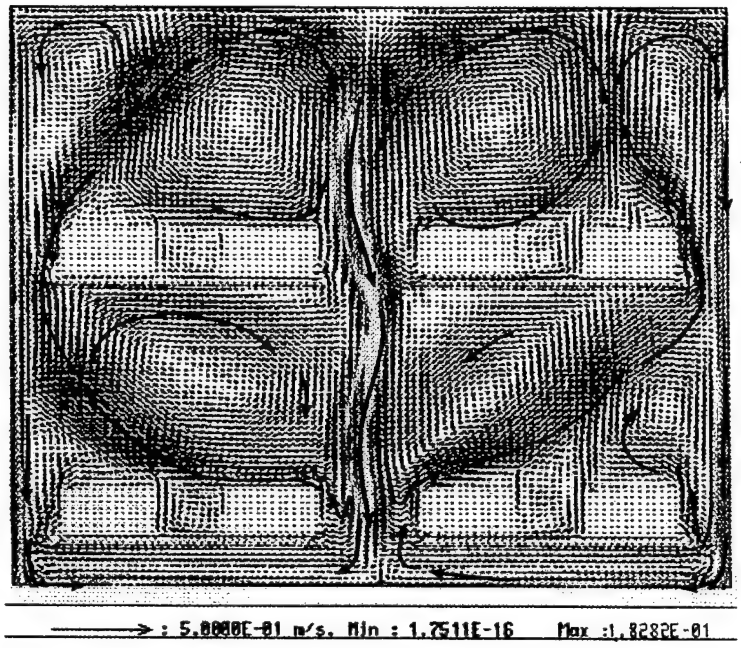


FIGURE 14. (*Continued*) (e) Chamber flow and thermal pattern with animals, t = +40 s. Temperature difference = 3.0°C, inlet = 27.0°C, end wall = 24.0°C. Velocities: inlet = 4.1×10^{-3}, minimum = 1.75×10^{-16}, maximum = 1.83×10^{-1} m/s.

the lower baffles, causing a centerline upward flow "opposed" to the upper core flow. This opposed upward core component influenced the oscillating downward core flow and subsequently the development of the lower set of Bénard cells, which in turn influenced the cyclic development of the upward centerline reflux flow. Asymmetry of the upper and lower baffles with respect to the centerline was simulated by shifting one numerical cell. This resulted in no marked changes in flow structure except for a slight lateral shift of the flow structure from the centerline.

DISCUSSION

Overall variation of concentration in the chamber (3.5–5.2%, depending on configuration) was acceptable for most inhalation study applications and compared favorably with exposure chambers of comparable design. As expected, excreta pans, acting as baffles, had effects on overall chamber performance, as was obvious from the appearance of piston flow at the expense of the fraction of well-mixed volume. Some local anomalies of concentration and variability could be attributed to the pans as well. The pans also, most likely, were responsible for the increase in spatial component contribution to total chamber variation. Various characteristic flow structure patterns readily correspond to the pattern of concentration anomalies observed in the whole chamber configured with cages and catch pans but without animal heat sources (Figure 6). The reflux flow structure observed for one thermal profile (Figure 10) corresponds well with the pattern of significantly lower concentration observed in specific cells of the middle sampling cell layer. Transition to another thermal profile (Figure 11) with significant upward reflux flow could account for the upper layer concentration anomalies observed for this chamber configuration. The asymmetry of the upper layer concentration differences was thought to be due to the fact that this side of the exposure chamber was located directly under an inlet flow register for the laboratory HVAC (heating, ventilation, air conditioning) system. Slight preferential cooling of one side of the chamber could account for the observed concentration asymmetry in the upper sampling tier. The third typical thermal gradient flow structure (Figure 13) corresponds well with observed concentration difference in one of the lower sampling cells. It is likely that during the course of the experimentation all three thermal gradient types and their characteristic flow structures were present and that transition between these thermal gradient patterns and flow structures occurred as the laboratory environment changed over the course of the investigation. These same flow structures could readily account for the observed cell variability differences (Figure 8).

Inclusion of animals in the chamber apparently affected chamber

distribution characteristics in a manner consistent with thermal and flow structures as predicted by the simulations (Figure 14). Local deviations of concentration in the top layer of the exposure volume, as occurred without animals, were not observed (Figure 7). It is speculated that constant formation, dissipation, and reformation of Bénard cells in the upper tier aided mixing and thus more uniform distribution of aerosol in this layer (compared to configuration 2), despite lack of a corresponding significant increase in well-mixed volume fraction (see Table 3). Bénard cell formation with incumbent vortices of static air and fluctuations in central core flow structure and velocity between the catch pans and catch pan tiers most likely accounted for the persistently lower concentration in certain middle layer sample cells. With animals in the chamber, the effects of different materials of chamber construction on chamber flow structure may have come into play. The metal rear wall of the chamber was more thermoconductive than the glass of the three other walls, introducing a front-to-back thermal gradient and flow structure on top of the thermally driven flow structure imposed by the animal heat sources. Heat transfer through chamber walls has been shown to be a significant source of thermal regulation in exposure chambers (Bernstein & Drew, 1980). This heat transfer, with implied additional accompanying convection currents, may have been responsible for the deviations of variability found only in all cells located next to the metal wall (Figure 9). Three-dimensional simulations that include a slight "front-to-back" thermal gradient showed that although the basic flow structure remained intact there was a corresponding velocity gradient imposed on the flow structure (Yerkes & Faghri, 1992).

There was a marked overall concurrence of chamber performance evaluation based on measurement and analysis of spatial concentration and variability with chamber performance analysis based on computational fluid mechanical analysis of chamber flow structure. In addition to the concurrence of spatial distribution analysis and flow structure determination, there also was a remarkable agreement between conventional measures of chamber mixing characteristics based on chamber filling (emptying) kinetics and estimation mixing in the chamber based on using fluid dynamic calculations of heat and mass transport in the chamber.

The structural modification of exposure chamber inlet and exhaust configuration from earlier versions served to reduce spatial variation of aerosol distribution within the chamber effective volume. It also demonstrated that, as expected, multitier configuration of an exposure chamber (with recommended excreta catch pans) causes a deterioration of chamber performance with respect to mixing and spatial distribution (Table 4). Comparison of chamber performance in different configurations demonstrated the impact of thermal effects on chamber flow dis-

tribution and flow structure. In the present investigation, thermal effects proved beneficial with respect to lowering overall chamber concentration variability (Table 4). However, a closer examination of the data demonstrated that thermal-gradient-driven changes in flow structure and dynamics tend to increase the number of cells that are highly variable and increase the magnitude of the variability within individual cells (Figure 9), and this is thought to be primarily a function of changes in basic flow structure not only within the cells but between cells.

Computational methods demonstrated that development of the flow structure within the chamber was found to be predominantly buoyant in nature and sensitive to thermal gradients between the inlet and walls. Flow structure behavior varied from steady-state to asymmetric oscillatory as a function of the magnitude and direction of the thermal gradient. Inclusion of the effects of mixed convection on heat and mass transfer and subsequently flow structure allows reliable estimation of chamber performance based on flow structure analysis that compares favorably with more conventional methods of assessing chamber performance. The forces governing the development of air flow structure in inhalation chambers can adequately be described in terms of three fluid parameters: Reynolds (Re), Grashof (Gr), and Prandtl (Pr) numbers. In the present investigation for the $\Delta T = 3°K$ case, the typical value of Re, the most fundamental parameter of fluid mechanics (White, 1986) characterizing the ratio of inertial to viscous effects, was 235. A typical Gr, characterizing the ratio of buoyancy to viscous effects and indicative of natural convection, was 3.21×10^8. A typical Pr, characterizing the ratio of dissipation to conduction effects and characterizing heat convection, was 0.7. The typical Rayleigh number (Ra), the product (Gr•Pr), was 2.25×10^8. Modeling of chamber flow structure with fluids other than air can be useful for understanding the effects of chamber configuration and structure on flow distribution. However, these models are not amenable to the investigation of thermal gradient effects on chamber flow structure. For example, using water as the fluid, similitude (with the already discussed case) of Gr would require a ΔT of $4.09 \times 10^{-2}°K$; however, similitude of Pr would require $T \cong 520°K$, conditions that cannot be satisfied.

Optimization of inhalation exposure chamber design has been, for the most part, an empirical exercise. Application of computational analysis of the development of flow structure in these chambers represents an additional tool for chamber design. Analysis of chamber flow structure and mass transfer forces (i.e., diffusion vs. convection) within that structure is crucial to an understanding of test material distribution and control of distribution within the chamber. A better understanding of exposure chamber flow structure development can be used as a guide for test material–specific retrofit or reconfiguration of the chamber

when necessary. Likewise, optimization of test material distribution within a chamber may be a matter of temporary modification of chamber operating parameters such as inlet velocity or thermal gradient. For example, preliminary findings of this investigation proved useful for optimization of toxicant delivery and distribution by manipulation of the thermal gradient in an exposure chamber (250 L, Wahmann Mfg, Timonium, MD) that had modifications similar to the THRU chamber design. Mass balance determinations in an investigation of the acute inhalation toxicity of *O,O'*-diethylmethylphosphonite (an agent subject to rapid hydrolysis in humid atmospheres; Kimmel et al., 1990) indicated that under routine operating conditions 92% of generator output was either lost in transit or adhered to the exposure chamber inlet plenum and walls and therefore was not airborne in the exposure volume. Placement and operation of a heating coil at 35°C in the plane of the exhaust manifold altered chamber flow structure sufficiently to effect a 10- to 11-fold increase in airborne test material concentration in the exposure volume, with an acceptable elevation of chamber operating temperature (20.6 ± 0.4 to 24.9 ± 1.9°C). Without this modification of chamber operating conditions the investigation would not have been possible. We believe that this investigation demonstrates that even when changes in chamber geometry and configuration result in improvement in chamber performance, there is a point where further improvement lies not in the realm of geometric and structural modification but possibly in the realm of thermal effects management. This investigation is an initial step toward evaluating the nature of these thermal effects.

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CHARACTERIZATION OF THE METABOLISM, DISTRIBUTION AND TOXICITY OF 2,6-di-*t*-BUTYL-4-NITROPHENOL FOR PURPOSES OF HEALTH HAZARD ASSESSMENT

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PREFACE

This is the first technical report that summarizes the findings of the research project entitled "Characterization of the metabolism, distribution and toxicity of 2,6-di-*t*-butyl-4- nitrophenol (DBNP) for purposes of Health Hazard Assessment." The research described in this technical report began in October, 1993 and was completed in April, 1995. This study was sponsored by the U.S. Navy under the direction of the Officer-in-Charge (OIC) of the Toxicology Detachment, Naval Medical Research Institute, CAPT David A. Macys, MSC, USN (the initiating OIC) and CAPT Kenneth R. Still, MSC, USN (the terminating OIC) and was supported by the Naval Medical Research and Development Command, Task No: 63706- M0096-004-1405. The scientific objective of this study was to gain toxicological information about DBNP. The research described in this technical report was carried out at the Naval Medical Research Institute Detachment (Toxicology) Bldg. 433 Area-B, Wright-Patterson Air Force Base, OH 45433-7903 by Navy, civil service, and contract scientists under the scientific supervision of CDR John Wyman, Ph.D., MSC, USN (Principle Investigator), and, upon his retirement from the Navy, Dr. Robert L. Carpenter, Ph.D. (Senior Scientist/Principle Investigator). The assistance of the following individuals during various phases of this research effort are gratefully acknowledged: J.A. Rivera, MS, for his analytical chemistry evaluation of DBNP samples and his development of a synthesis procedure for DBNP; HM1 C. Alva, USN; and HM3 D.L. Lee, USN for their untiring assistance in the laboratory during the rat liver perfusion experiments.

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, Department of Health and Human Services, National Institute of Health Publication # 86-23, 1985, and the Animal Welfare Act of 1966 as amended.

The opinions expressed herein are those of the authors and are not to be construed as official or reflecting the views of the Department of the Navy or the Naval Service at large.

LIST OF TABLES & FIGURES

ABBREVIATIONS

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
C-18	Carbon-18
DBNP	2,6-di-t-butyl nitrophenol
DBP	2,6-di-t-butylphenol
DMSO	Dimethyl sulphoxide
DPM	Disintegration Per Minute
DSC	Differential Scanning Calorimetry
HPLC	High Performance Liquid Chromatography
i.p.	Intraperitoneal
IR	Infra Red
i.v.	Intravenously
LD-50	Lethal Dose-50
NMR	Nuclear Magnetic Resonance
NO_2	Nitrogen Dioxide
RP	Reverse Phase
TCA	Trichloroacetic Acid
TGA	Thermal Gravimetric Analysis

ABSTRACT

In 1992, the Navy Environmental Health Center, Norfolk, VA was made aware of the concern about the discoloration (yellowing) of interiors (e.g. bulkheads and bedding) and the possible exposure of Navy personnel aboard submarines, to an unknown substance. The agent was identified as 2,6-di-tert-butyl-4-nitrophenol (DBNP). The yellowing process appeared to arise from the reaction of 2,6-di-tert-butylphenol (DBP), an antioxidant additive used in engine lubricant, with NO_2 in the submarine atmosphere. A research program was initiated for health hazard assessment of DBNP. This technical report summarizes the results of our research program and the information available in the literature. The LD-50 dose by intraperitoneal route in rodents is above 250 mg/Kg. DBNP is as half toxic by oral route and no sign of toxicity or skin irritation at high doses applied dermally. Even though these acute toxicity tests demonstrated that DBNP has a low toxicity, tissue distribution, metabolism and excretion studies carried out in rats clearly suggests, that DBNP has considerable tendency to produce cumulative toxic effects at low doses due to slow excretion and storage in fats. At the cellular level the toxicity expressed by DBNP is very likely due to its inhibitory effects on ATP synthesis.

SECTION 1

INTRODUCTION

BACKGROUND

This technical report is the final report summarizing the research activities executed by the Naval Medical Research Institute Detachment Toxicology (NMRI/TD) under work unit NMRDC Task No. 63706-M0096-1405 and serves as a final activity report for that work unit. The scope of research is the result of a collaborative effort to address rapidly the emerging submarine fleet toxicity issues involving NMRI/TD, the Navy Environmental Health Center (NEHC) and NAVSEA engineering personnel (Code 390).

This work unit was originally conceived to develop advanced methods of detecting liver toxicity resulting from exposure to toxic chemicals. However, in March of 1992, NEHC received inquiries concerning a yellow discoloration of bulkheads aboard submarines which occurred while they were underway. Subsequent investigation suggested that the causative agent was 2,6-di-*t*-butyl-4-nitrophenol (DBNP) produced as a result of nitration of a phenolic additive to engine lube oil (2,6-di-*t*-butylphenol, DBP) which was presumably released into the air during operations. The findings leading to this hypothesis are reviewed in Appendix A. NEHC contacted NMRI/TD with a request for toxicity data on DBNP. Review of the toxicology literature (see below) lead to the conclusion that additional toxicity study was warranted, and DBNP was chosen as the test agent for this work unit. Work unit goals were modified to include toxicity evaluation of DBNP.

EXISTING TOXICOLOGY DATA

DBNP was originally evaluated for use as a mitocide but was found to have low acute toxicity to mammals (Vesselinovitch *et al.* 1961). Acute toxicity studies carried out in rats,guinea pigs and mice demonstrated that this compound has low toxicity (i.p. LD_{50} 270 mg/kg for rats; 580

mg/kg for guinea pigs, and 700 mg/kg for mice). Daily administration exceeding one twenty-fifth of the LD_{50} dose in rats produced 40% mortality. Holder *et al.* (1971) reported that after i.p. injection, DBNP is excreted in the urine, feces and a small amount in the bile. Orally administered DBNP was poorly absorbed from the gut (30% excreted unchanged) but, once absorbed, was excreted as a glucuronide conjugate. No other metabolites were detected.

NMRI/TD EVALUATION

Apart from the two papers published be Vesselinovitch *et al.* and Holder *et al.*, there is little information concerning the toxicity and mechanism of action of DBNP. This study was undertaken to evaluate the toxicity of DBNP and, if possible, provide clinical parameters that could be routinely monitored as part of medical surveillance of DBNP-exposed submariners. The present investigation was undertaken to study the metabolism, tissue distribution and tissue specific toxicity of DBNP in Fischer-344 rats. Metabolism and tissue distribution studies were carried out using ^{14}C-DBNP (ring-labeled). Toxicity studies were carried out both *in-vitro* and *in-vivo* using tissue slices and a hepatocyte cell line in culture. Biliary excretion studies were performed using a rat liver perfusion procedure. This report summarizes the work carried out in our laboratories and that from the literature.

SECTION 2

MATERIALS AND METHODS

ANIMALS

Upon receipt from the Charles River Breeding Labs (Raleigh, NC) male and female Fischer-344 (F-344) rats, weighing 200-250g, were quality control tested prior to use in the studies. Rats were individually housed in stainless steel wire-mesh cages with water and feed (Purina Rat chow # 5008) available *ad libitum*. The vivarium in which the animals were housed is maintained at 21 to 25 ° C with 12-h light/dark cycle (light cycle start at 0700 hours). Once the control (vehicle alone) and experimental (DBNP) groups were treated, they were transferred to individual plastic (Nalgene) metabolic cages. All rats were identified by tail tattoo. Scientists involved in the handling of animals underwent a yearly animal handler's medical check-up as well as attended the video tape demonstration and lecture on the care and use of laboratory animals mandated by the Animal Care and Use Committee.

CHEMICALS

All the chemicals used in this study are of analytical grade and the solvents are of HPLC grade. They were purchased from the Sigma Chemical Co. (St. Louis, MO) or from the Fisher Scientific Co. (Pittsburgh, PA). Radioactive ^{14}C-DBNP (ring labeled) was custom ordered from the Sigma Chemical Co.

Handling of radioactive chemicals and the disposal of radioactive wastes were in strict accordance with NRC guidelines governed by the Wright-Patterson Air Force Base radiation safety officer.

DBNP SYNTHESIS

A clear pale yellow solution of 2,6-di-*t*-butyl phenol (DBP Fig.1; Sigma Chemical Co.) was prepared in hexane (106g of DBP in 400 ml of hexane) with gentle stirring. A fritted glass tube was connected to an NO_2 gas cylinder (99.5% pure; Matheson Gas Company) using polypropylene tubing, and NO_2 was bubbled through the stirred solution at the rate of 500 ml/minute at ambient temperatures for an hour. Beige crystals began to appear approximately 20 minutes after bubbling of NO_2 began and the precipitation was completed within 1 hour. The solid was filtered and washed three times with 50 ml of hexane. The washed crystals were placed in (400 ml) boiling hexane (69° C) and sonicated for 10 minutes to enhance dissolution. Fine needles recrystalized after cooling the solution to room temperature. The crystals were filtered from the mother liquor and vacuum dried. Physical and spectral characteristics of the purified DBNP crystals were determined by doing: elemental analysis; X-ray crystallography; Thermal Gravimetric Analysis (TGA); Differential Scanning Calorimetry (DSC); GC-MS spectrophotometry; UV-VIS spectrophotometry; Fourier-Transformed ^{1}H-NMR ; Fourier - Transformed ^{13}C-NMR ; and Fourier-Transformed IR spectrophotometry (J.A. Rivera-Nevares *et. al.* 1995).

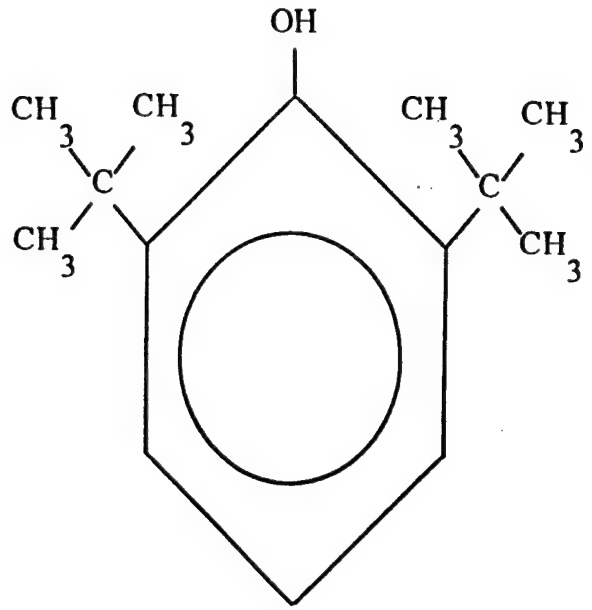
TOXICITY STUDIES

Cold DBNP stock: 10 mg/ml in 80% DMSO, pH adjusted to 7.4 with 0.5% $NaHCO_3$

Radioactive (^{14}C)DBNP stock: 0.456 mg/10 μCi/ml in 80% DMSO pH adjusted to 7.4 with 0.5% $NaHCO_3$.

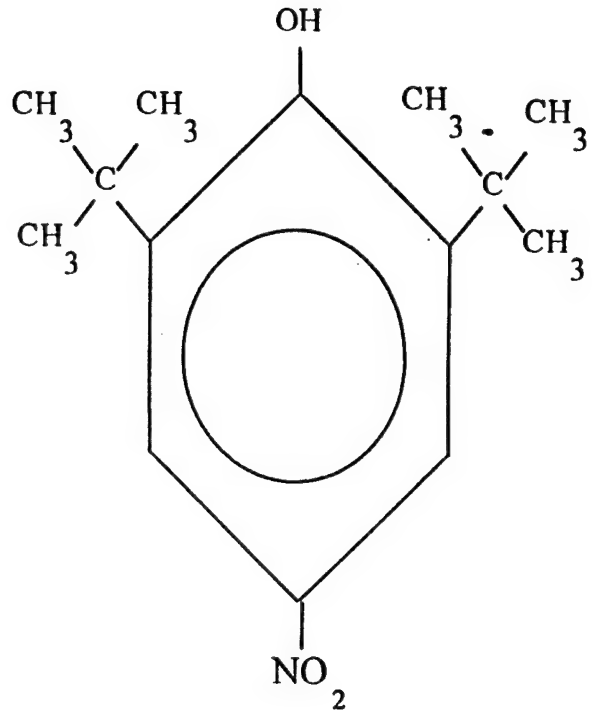
Rats (4 male) were administered with cold DBNP i.p. (10 mg/kg dose) for 10 days. The control group (4 male) received the vehicle alone (80% DMSO with pH adjusted to 7.4 with 0.5% $NaHCO_3$). Both the control and experimental groups were monitored for water consumption, urine and feces production and body weight for 10 days. All of the rats were closely monitored for abnormal changes in behavior.

Figure 1.



2, 6-bis (1,1-dimethylethyl) Phenol

Figure 2.



2, 6 di-t-butyl-4-nitrophenol

CLEARANCE OF DBNP FROM THE BLOOD

The rate of clearance of ^{14}C-DBNP from the blood was measured in male F-344 rats (200-250 g) following administration of 2μ Ci of DBNP (200 μl volume and 91.2 μg of DBNP/rat by i.p., i.v. [tail vein], and oral routes). After the administration of DBNP, a small sterile cut was made in the mid portion of the tail with the surgical blade, and 20 μl of blood was collected at each time point. Each sample was counted in a PACKARD liquid scintillation counter with 10 ml of cocktail (Scintiverse-Fisher Scientific Co. Pittsburg, PA). Quenching corrections were made, and the amount of radioactivity in the sample was expressed in DPMs. At the start of the experiment, blood was withdrawn from the tail cut at 10-minute intervals for an hour and was followed by 15-minute intervals for the next hour. During the third and fourth hours, samples were drawn at 30-minute intervals. From fifth hour onward, samples were taken hourly. At the end of the experiment, the rats were sacrificed, and the amount of radioactivity in different tissues was quantitated (see details below).Three rats were used for each route of administration.

EXCRETION OF DBNP IN THE URINE AND FECES

Rats were administered with ^{14}C-DBNP (2μCi/rat i.p.) and transferred to plastic metabolic cages for the collection of the urine and feces. Feces and urine were collected every 24 hours for 10 days. The urine volume was measured and aliquots (500 μl) were counted for radioactivity. The feces were weighed and homogenized with cold normal saline (10ml/g of feces) using a Polytron-tissue homogenizer(Brinkmann Westbury, NY) An aliquot (1ml) was counted for radioactivity.

TISSUE DISTRIBUTION OF DBNP

Rats were administered with ^{14}C-DBNP (2 μCi/rat i.p.) and sacrificed after 24 hours. The liver, kidney, spleen, heart, brain and fat were dissected, weighed, and homogenized with cold normal saline (10ml/g of tissue) and an aliquot was taken for counting.

ISOLATION, PURIFICATION AND IDENTIFICATION OF DBNP METABOLITE(S) FROM THE URINE AND FECES

Three rats were administered with ^{14}C-DBNP (2μCi / rat i.p) and the urine and feces from each rat was collected and pooled. The urine and feces samples were processed separately. The pooled urine was centrifuged at 10,000 x g for 20 minutes in a Sorval RC-5B, and the supernatant was collected and lyophilized. The lyophilized material was dissolved in a minimal amount of water and treated with powdered activated charcoal (100mg/10 ml) overnight with gentle stirring at 4° C. After charcoal treatment, the samples were centrifuged at 20,000 x g for 30 minutes. The pellet was washed with water (three to four times) until there was no radioactivity present in the supernatant. The supernatants from each centrifugation were pooled and lyophilized. The lyophilized material was dissolved with a minimal amount of water and loaded onto three C-18 bond-Pak columns arranged serially. The C-18 bond pack column was primed with methanol followed by water washing before the samples were loaded. After loading the samples, the column was washed extensively with water, and all of the eluent was pooled and lyophilized. The lyophilized material was dissolved in a minimal amount of water and applied to an HPLC column.

The pooled feces were homogenized with water (10ml/g of feces) using a Polytron homogenizer and centrifuged at 10,000 x g for 20 minutes in a Sorval RC-5B centrifuge. The supernatant was saved, and the pellet was washed three times with water followed by centrifugation. All of the supernatants were pooled and lyophilized. Further processing of the feces samples was done by the same procedures as described for the urine.

Subsequent purification of the sample was carried out using a Beckman HPLC Gold System. Separation of the DBNP and its metabolite(s) was achieved using a C-18 reverse phase column (LiCheosphere 100 RP-18 endcapped (5 μm); Column length -250 mm; Internal Diameter- 4 mm Hewlett-Packard, USA). The separating column was protected with a C-18 guard column. Samples were loaded onto the column (55min/run) in an aqueous phase and maintained in that phase for 5 minutes using 100 % water, and a linear gradient of methanol from 0 to 100 % was achieved in 15 minutes. The column was maintained in 100% methanol for 5 minutes and reverted back to 100 % water at the end of the run.. The peaks were detected with an on-line UV-VIS spectrophotometer and by a radioisotope detector. The radioactive peaks with the same retention times were pooled, concentrated, and rerun on the HPLC with the same eluting conditions to check the purity. In each purification step, a small aliquot was counted to calculate the percentage of recovery.

IN-VITRO LIVER PERFUSION

Isolated perfused liver was prepared according to the method of Miller *et. al.* (1951) with minor modifications. All the surgical procedures were carried out under aseptic conditions. The rat was anesthetized with ether, and the liver was exposed. The common bile duct was cannulated with PE-10 tubing. The animal was heparinized with 1000 units of sodium heparin injected through the inferior vena cava. Immediately after the injection, the inferior vena cava was ligated anterior to the site of injection. The portal vein was cannulated with PE-240 filled with the perfusate. The outflow cannula was inserted into the right atrium, and the liver was removed with the diaphragm and immediately placed in a container of warm saline. This entire setup was placed in a humidified atmosphere. The liver was perfused with Krebs-Henseleit-Ringer [(NaCl-(118 mM); KCl-(4.7 mM); $CaCl{\cdot}2H_2O$-(10 mM); $MgSO_4{\cdot}7\ H_2O$;(1.2 mM); KH_2PO_4 -(1.2 mM); $NaHCO_3$ -(25mM); dextrose- (11.5 mM); pH 7.4] at the rate of 25ml/minute at 37°C with 95-5% O_2-CO_2 . The perfusate was supplemented with 5 mM sodium taurocholate to maintain the bile flow throughout the experiment. After an equilibration time of 30 minutes,

2μCi of ^{14}C-DBNP was added to the perfusion fluid, and the bile was collected. The effluent from the outflow cannula was reefed to the reservoir. The perfusion of the liver with DBNP in the closed system was carried out for 2 hours. The viability of the liver was monitored during the perfusion period by measuring the oxygen consumption. The oxygen consumption by the liver was calculated by measuring the oxygen tension in the perfusate before it enters the liver and after it effuses from the liver. This was repeated four times, and the bile was pooled and analyzed for the metabolite(s).

EFFECT OF DBNP ON HUMAN AND RAT LIVER SLICES

This study was carried out by VITRON, INC. Tucson,AZ 85747. Human liver tissue was obtained from the Association of Human Tissue Users (Tucson, AZ). The liver was procured for transplantation by organ banks but was not used for transplantation for various medical reasons. Once it was decided that the tissue would not be suitable for transplantation, it was immediately placed in an ice-cold solution of Viaspan (a cold preservation solution from DuPont Pharmaceuticals [Wilmington, DE]). Tissue slices were prepared both from human and rat tissue using a Brendal/Vitron tissue slicer and kept in V-7 preservative medium (Tucson, AZ). The slices (200μm) in V-7 preservation medium were floated onto Teflon®/vitron/titanium rollers. The rollers were then carefully blotted and loaded horizontally into glass scintillation vials containing 1.7 ml of Waymouth's culture medium which had been supplemented with 10% fetal calf serum (Hyclone Laboratories), 10 ml/l Fungi-Bact, 84μg/ml of gentamicin, 3.5 mg/ml L-glutamine, and 2.4g/l sodium bicarbonate. Vials were closed with a cap which had a central hole of approximately 2mm, placed in the dynamic organ culture incubator and gassed with 95%-5% O_2/CO_2 mixture. The liver slices were acclimatized for period of 1 hour prior to exposure to individual nitrophenols. Nitrophenols, in an amount required for a specific concentration, were dissolved in 100 μl of DMSO and added to the incubation medium. Control exposures contained 100μl of DMSO alone in the medium. Measurement of intracellular K^+, protein synthesis, LDH leakage and ATP content were used as markers of toxicity.

Intracellular K^+ content

At the end of the incubation period, the slices were removed and homogenized in 20 µl of cold 70% perchloric acid followed by centrifugation. The supernatant was assayed for K^+ using a Model CA-51, Perkin-Elmer Flame Photometer. Results are expressed as µmol of K^+/g wet weight.

Protein Synthesis

Slices were exposed to ^3H- leucine for a specific period of time. At the end of the exposure, the slices were removed and washed three times with the buffer. The washed slices were homogenized with 15% ice-cold TCA followed by centrifugation. The precipitated protein was washed three times with ice-cold TCA and counted in a liquid scintillation counter. Results were expressed as DPM/mg wet weight.

LDH Leakage

Lactate dehydrogenase activity in the incubation medium was measured using an LDH assay kit from Sigma Chemical Co.

ATP Content

ATP content was measured in the TCA homogenate using a luciferin-luciferase assay kit purchased from Sigma Chemical Co.. Results are expressed as nmol ATP/mg wet weight.

EFFECT OF DBNP ON THE HEPATOCYTE CELL LINE

WP-344 cells are routinely maintained in Gibco MEM medium supplemented with 10% fetal calf serum from Hyclone Laboratories. Confluent cells in 100 mm plates were washed once

with Gibco MEM medium (no serum) and exposed to DBNP (0.1μg-2μg/ml) for 16 hours. The control cells were exposed to 5μl of 80% DMSO (adjusted to pH 7.4 with 0.5% $NaHCO_3$). At the end of the incubation period, the cells were counted for viability by the tryphan blue dye exclusion method with hemocytometer counting.

MITOCHONDRIAL RESPIRATION

Mitochondria were prepared from rat liver by the following method. Rats were decapitated, and the liver was quickly removed and placed in an ice-cold 0.25M sucrose in 0.07M Tris-HCl buffer pH 7.4 (10ml buffer/g tissue). After the liver was minced into small pieces, it was homogenized using a Potter-Elveign homogenizer (Brinkmann, Westbury, NY). The homogenate was centrifuged at 2500 x g for 10 minutes. The supernatant was saved and centrifuged at 10,000 x g for 10 minutes. The crude mitochondrial pellet was washed three times with Tris-HCl sucrose buffer.

Isolated mitochondria were placed into an 1-3 ml oxygen electrode cell with a stirrer containing the reaction mixture. The mixture was composed of 40 mM Tris-HCl pH 7.5; 5 mM K_2HPO_4; 5 mM Mg SO_4 and 100 mM KCl with 2 mg of mitochondrial protein/ml.

A stable recorder baseline was obtained before the initiation of state-4 respiration by adding succinate to yield a final concentration of 5 mM. After 1-2 minutes of state-4 respiration, state-3 respiration was initiated by adding 5μ moles of ADP. The effect of DBNP on state-4 and state-3 mitochondrial respiration was compared with a known uncoupler of mitochondrial oxidative phosphorylation, DBP.

EFFECT OF DBNP ON RAT LIVER FATTY ACID BINDING PROTEIN AND RAT LIVER SULFOTRANSFERASES

This study was carried out by Dr. Sanford S. Singer Ph.D at University of Dayton, Dayton ,OH. Fatty acid binding protein (FASB) and sulfotransferases which includes bile acid

sulfotransferase (BST), dopamine sulfotransferase (DST), cortisol sulfotransferase (HCST) and estrogen sulfotransferase, were prepared from rat liver cytosol using the standard procedure. The effect of DBNP (200 μ M) on FABP protein was measured *in-vitro* using rose bengal assay method.. Sulfotransferases (BST, DST, HCST and EST) were routinely assayed using the corresponding substrate (bileacid, dopamine, cortisol and estrogen) in the assay mixture containing radioactive coenzyme 3' - phosphoadenosine-5'-phosphosulfate (PAPS- ^{35} **S**). The effect of DBNP (200 μ M) was measured on all four sulfotransferases. FABP and sulfotransferases activity were also determined in rat liver perfused with DBNP(0.36 mM) and also in liver of rats administered with DBNP (25mg/kg, i.p. for 60 days).

SECTION 3

RESULTS AND DISCUSSIONS

The reaction conditions for the synthesis DBNP from DBP are shown in Fig .3. Under these conditions, the yield was 75%. The elemental analysis of the purified product showed the chemical composition as being 67.09% carbon, 8.16% hydrogen, and 5.49% nitrogen. The theoretical values for DBNP are 66.95% carbon, 8.36% hydrogen, and 5.58% nitrogen. HPLC analysis of the purified product by Sigma Chemical Co. indicated a purity of 99.5%.
The single crystal structure of DBNP grown in hexane is shown in Fig.4. DBNP crystallizes in the acentric orthorhombic space group $Pna2_1$. The nitro group is virtually coplanar with the aromatic ring and exhibits an O2-N-C4-C3 torsional angle of 0.4(6)° . The hydroxy- group hydrogen H1 is rotated from the plane of the aromatic ring to exhibit the C6-C1-O1-H1 torsional angle of -17(4)° . An intermolecular hydrogen-bonding interaction was found between the hydroxy-group hydrogen H1 of one DBNP molecule and nitro-group oxygen O2 of an adjacent one in an head-to-tail arrangement.

The Thermal Gravimetric Analysis (TGA) shows that DBNP begins to lose its mass at 125°C and achieves its fastest rate of mass loss at 177.83°C. It lost 100% of its original mass at about 212°C.
The DSC thermogram analysis of DBNP showed two endotherms. One occurred at the extrapolated onset of 157.8°C (ΔH_1 = 123.5 J/g) and the other, after cooling and reheating, at the extrapolated onset of 152.49°C (ΔH_2=85 J/g). Thus, the data suggest that two well-defined crystalline domains exist for DBNP . The proposed existence of two stable packing configurations is further supported by the typically encountered discrepancy in the melting point profile reported for DBNP (152 to 153°C *vs* 156 to 157 °C).
The physico-chemical properties of DBNP are: a yellow powder; a melting point of 157°C;

Figure 3. Synthesis of DBNP

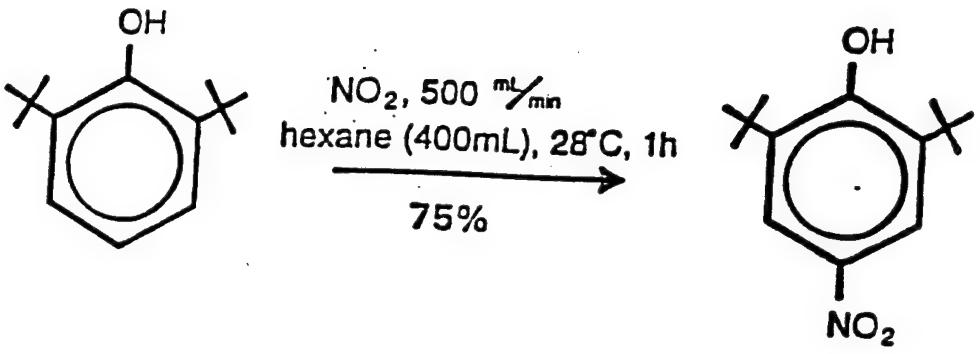
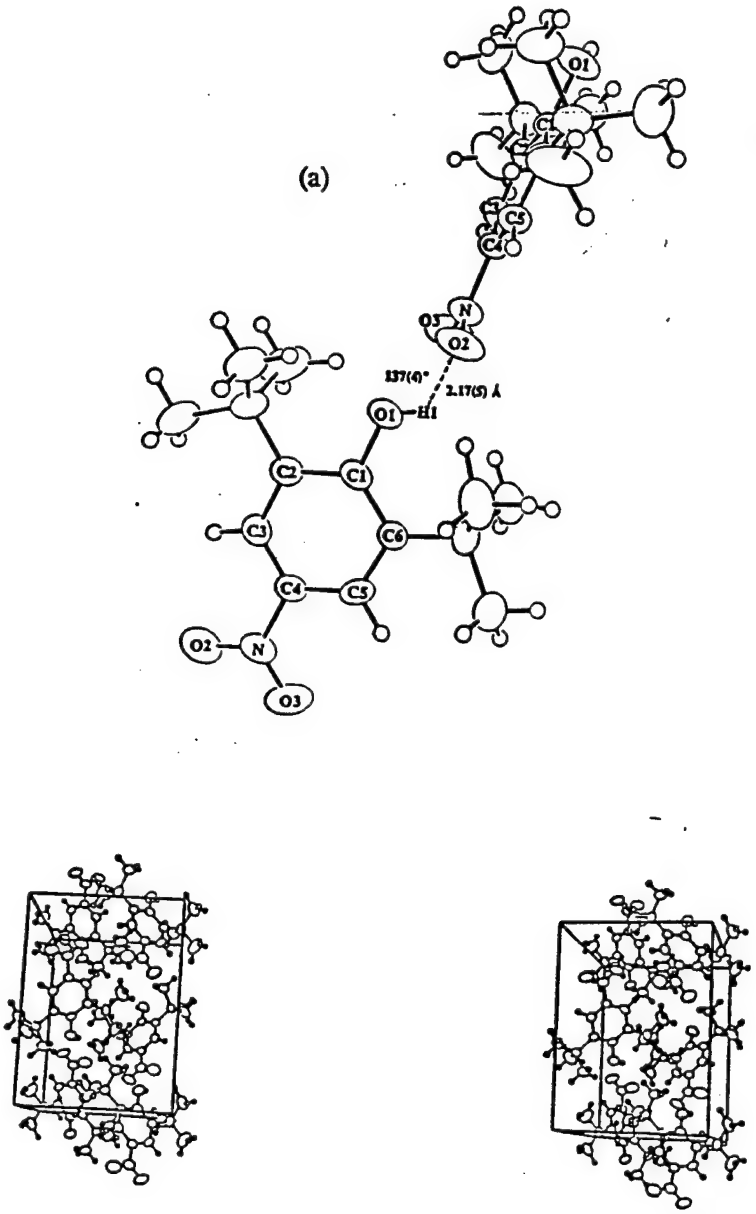


Figure 4. X-ray Crystal Structure of DBNP



soluble in organic solvents such as methanol, methylene chloride, hexane, acetone, benzene and ethanol; insoluble in water; soluble in aqueous alcohol, alkalinized water, and aqueous DMSO. DBNP has a λ_{max} of 300 nm in hexane and a λ_{max} of 320 nm in methanol and methylene chloride (Fig.5). The λ_{max} of DBNP in alkaline solution is 452 nm. The molar extinction coefficient (ε) of DBNP in organic solvents (ε_{320} = 10,092 $cm^{-1}.M^{-1}$) is threefold lower as compared to ε_{452} = 30,507 $cm^{-1}. M^{-1}$ in alkaline media. The λ_{max} at 452 is used for quantitation (Fig. 5).

Acute toxicity studies carried out by Vesselinovitch *et. al.* (1961) are summarized in Table 1. In all cases, the LD-50 is above 250 mg/kg. The oral dose is half as toxic as intraperitoneal administration in rats and guinea pigs. There is no sex difference, but rats are more sensitive to DBNP than mice or guinea pigs.

Daily administration of 10 mg/kg intraperitonealy for 60 days showed no significant effects on growth rate or on the mortality rate. During a sixty-day study, 20 mg/kg dose intraperitonealy, there was a 40% mortality, and the survivors showed decrease body weight. At a 50 mg/kg dose for sixty days, there was 100% mortality.(Vesselinovitch *et.al.* 1961)

DBNP mixed with the diet (Rockland rat diet) at 0.05% and 0.1% concentrations for 16 weeks did not show any significant effect on the growth rate. At 0.2%, DBNP half of the animals died during the first three weeks, and the survivors, both male and female, showed a significant reduction in their growth rate. DBNP in the diet caused a 25% reduction in food consumption.(Vesselinovitch *et.al.* 1961)

DBNP applied on the shaved skin of the rats (1000 mg/Kg) did not show any systemic toxicity, no mortality, nor any evidence of skin irritation.(Vesselinovitch et.al. 1961)

The effect of DBNP (10 mg/Kg, for 10 days i.p.) on the growth rate in male rats are shown in Fig. 6. There is no significant difference in growth rate between the control and treated groups.). The ratio of urine production to water intake remains the same in both the experimental and control group (Fig.7).

The clearance of ^{14}C-DBNP from the blood, administered through i.p., i.v., and oral routes is shown in Figs. 8, 9, and 10. DBNP reached a peak blood level in 5-10 minutes when administered i.p. as compared to about an hour in the case of oral administration. The rapid

TABLE 1 Acute toxicity studies of DBNP

Species	Sex	Routes of Administration	LD_{50} (mg/Kg)
Rat	Male	ip	260
	Female	ip	270
	Male	oral	450
	Female	oral	500
Guinea Pigs	Male	ip	580
	Male	oral	800
Mice	Female	ip	850
	Male	ip	700

Vesselinovitch *et al.* 1961.

Figure 5. Spectal Characterization of DBNP

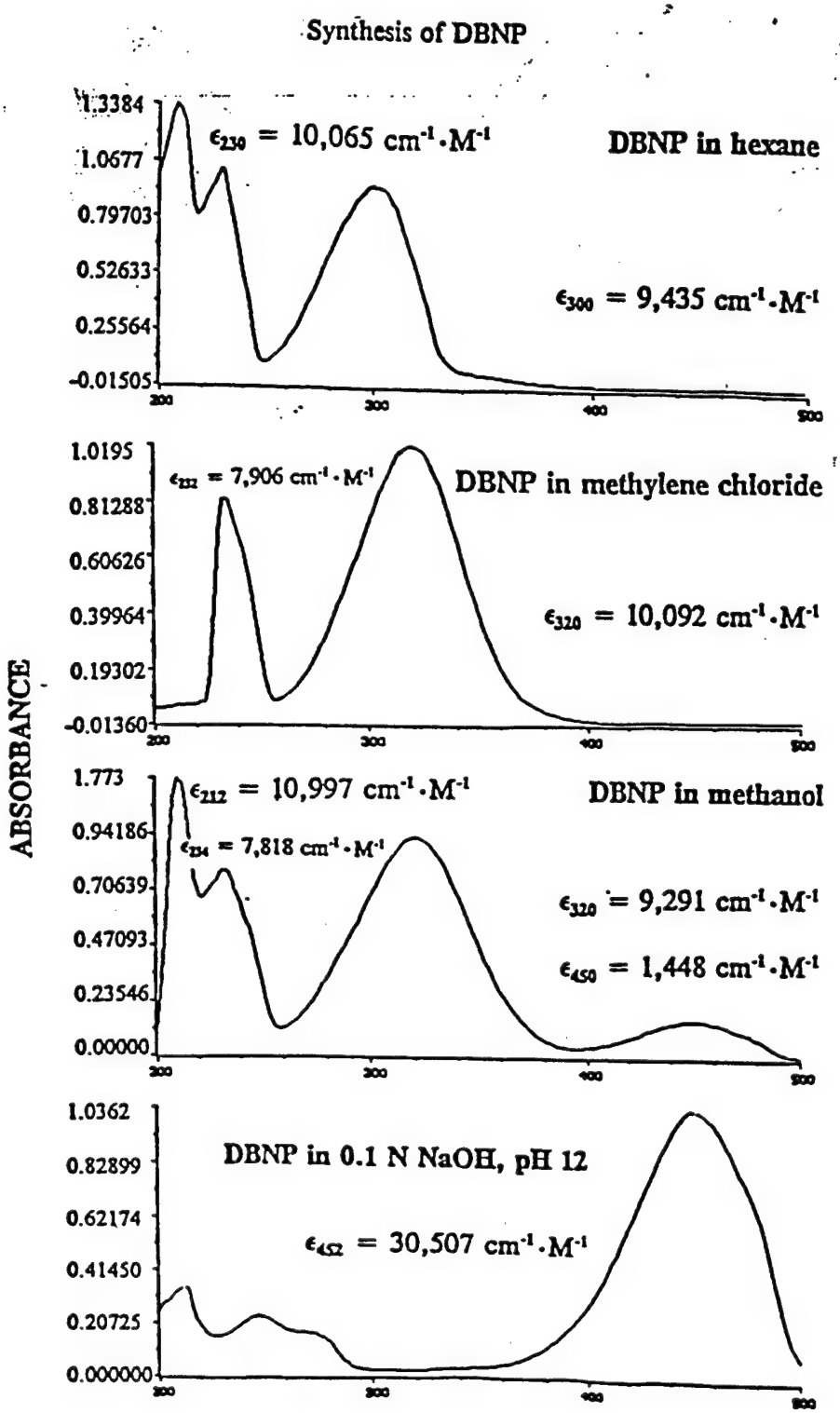


Figure 6.

Influence of DBNP on rat weight

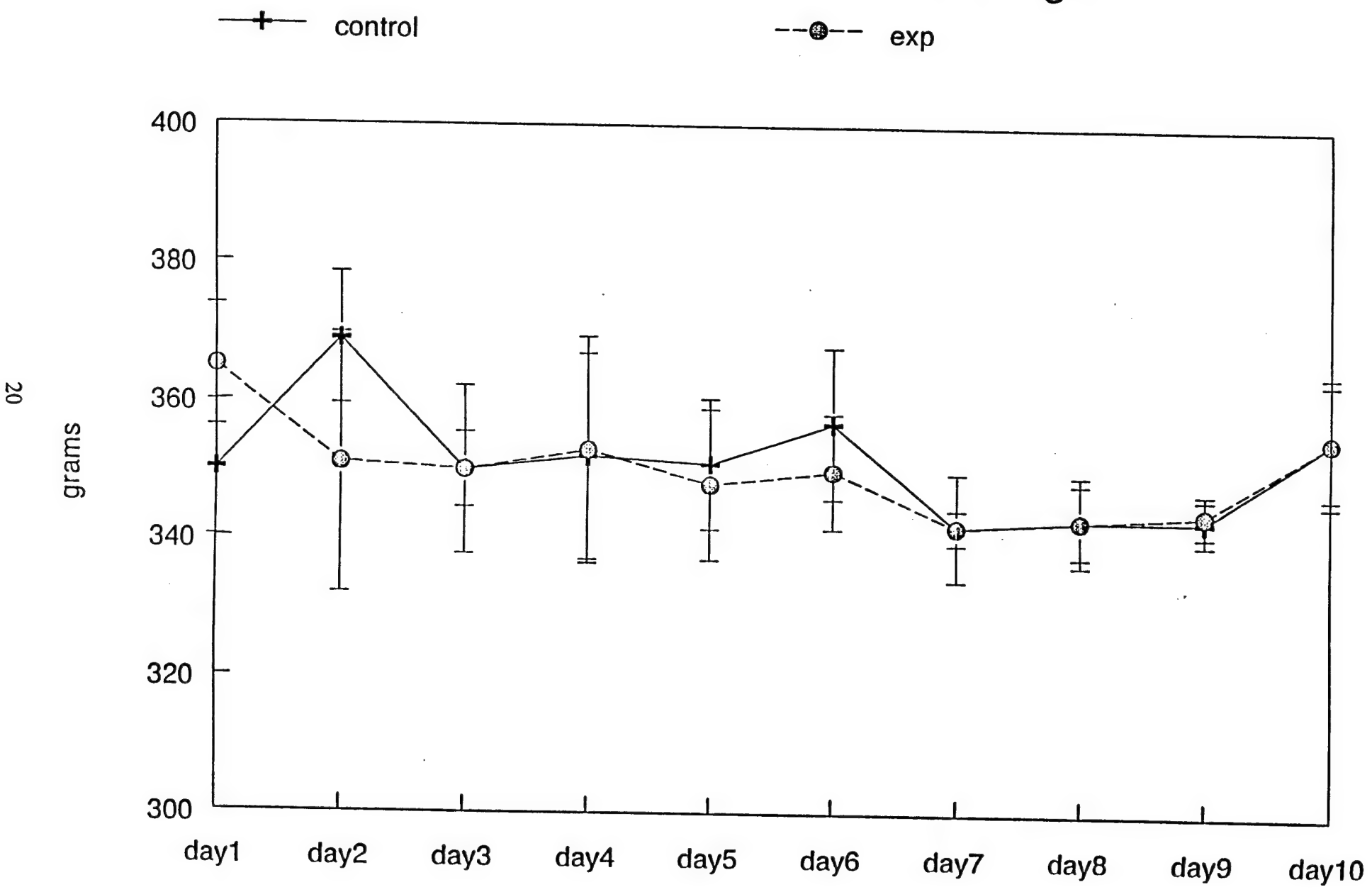


Figure 7.

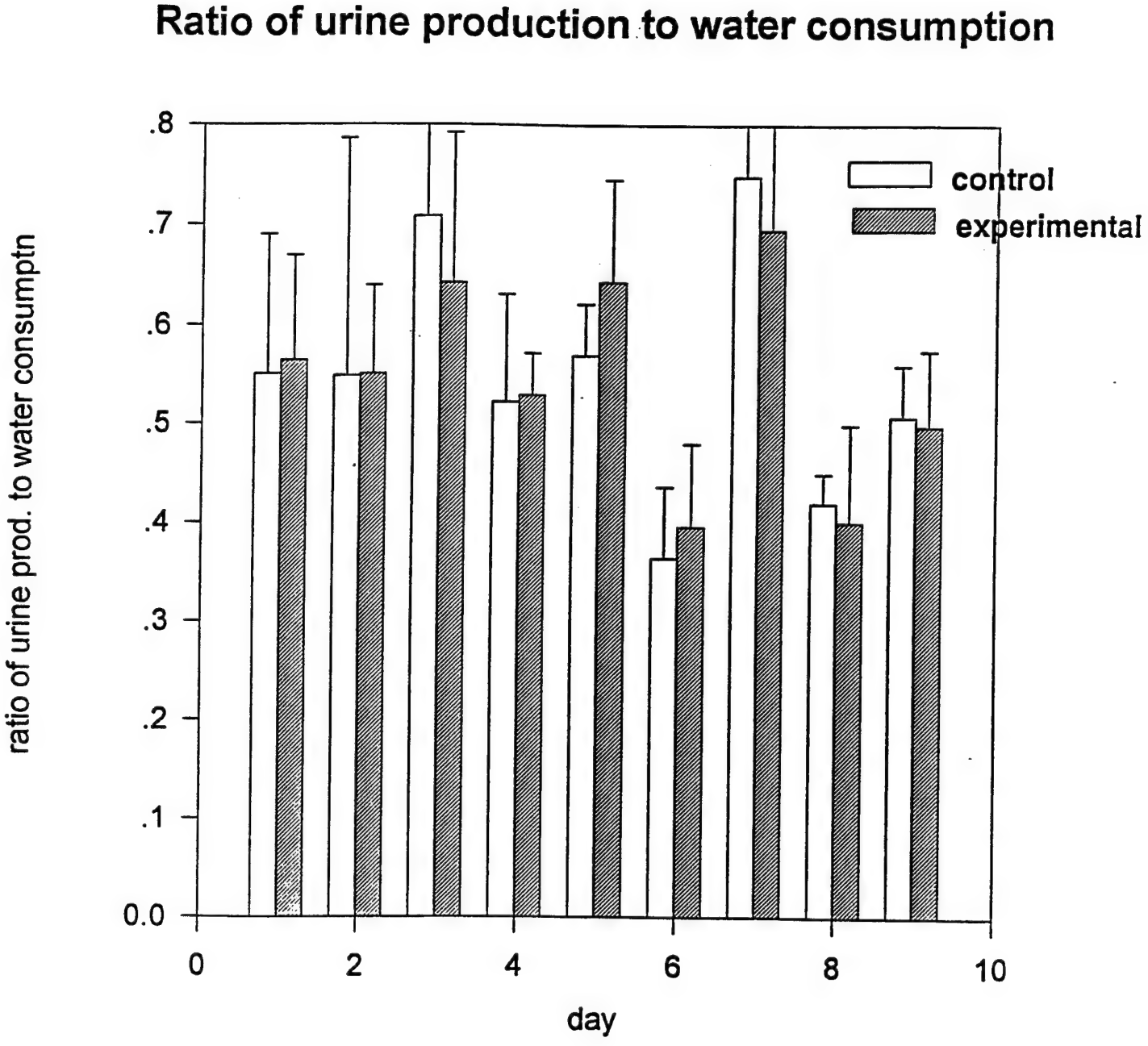

Ratio of urine production to water consumption
ratio of urine prod. to water consumptn
.8
.7
.6
.5
.4
.3
.2
.1
0.0
0
2
4
6
8
10
day
control
experimental

Figure 8.

Clearance rate of 14-C DBNP(i.p.) from the blood in 16 hou

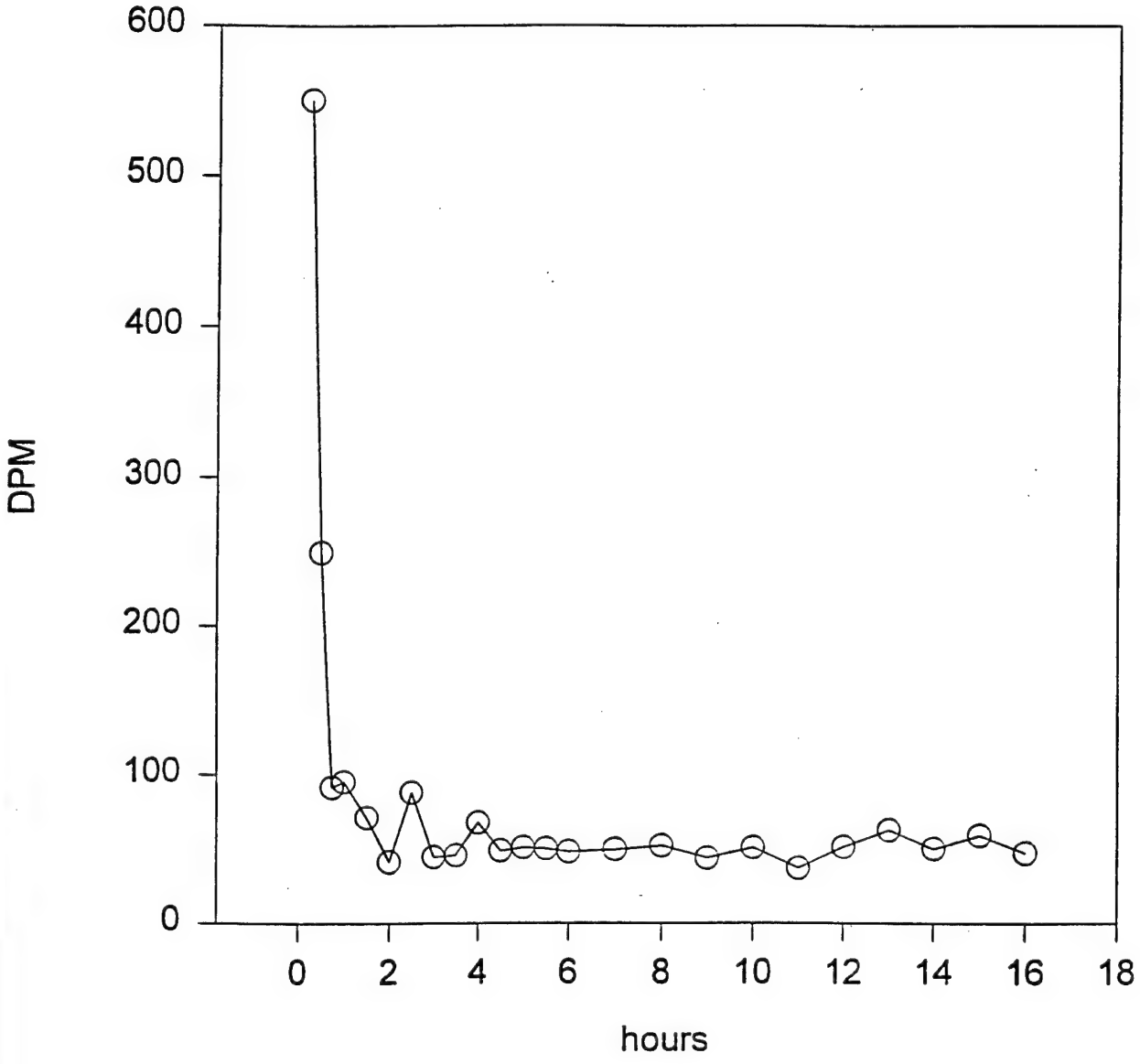


Figure 9.

Clearance of DBNP from blood, i.v.

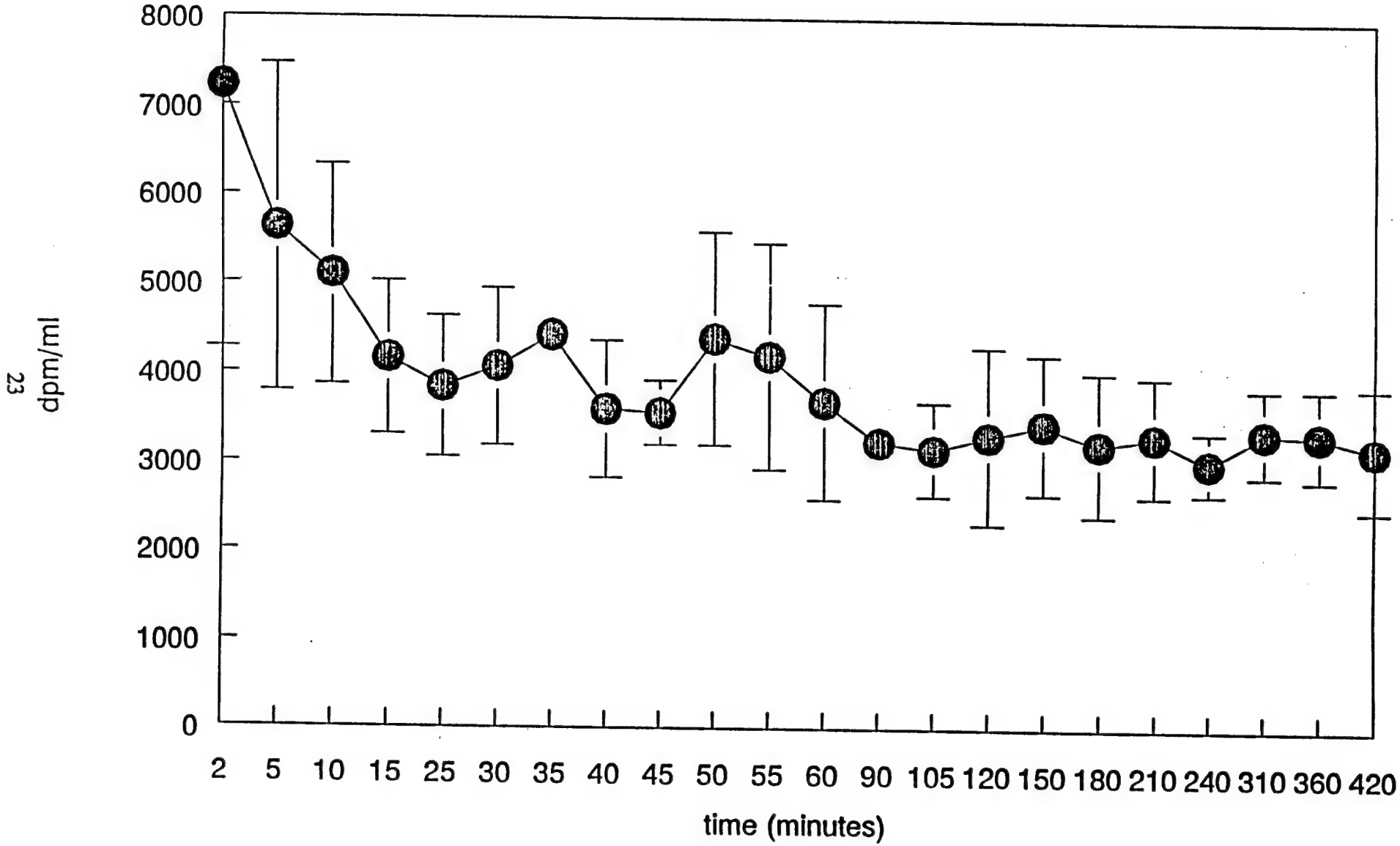
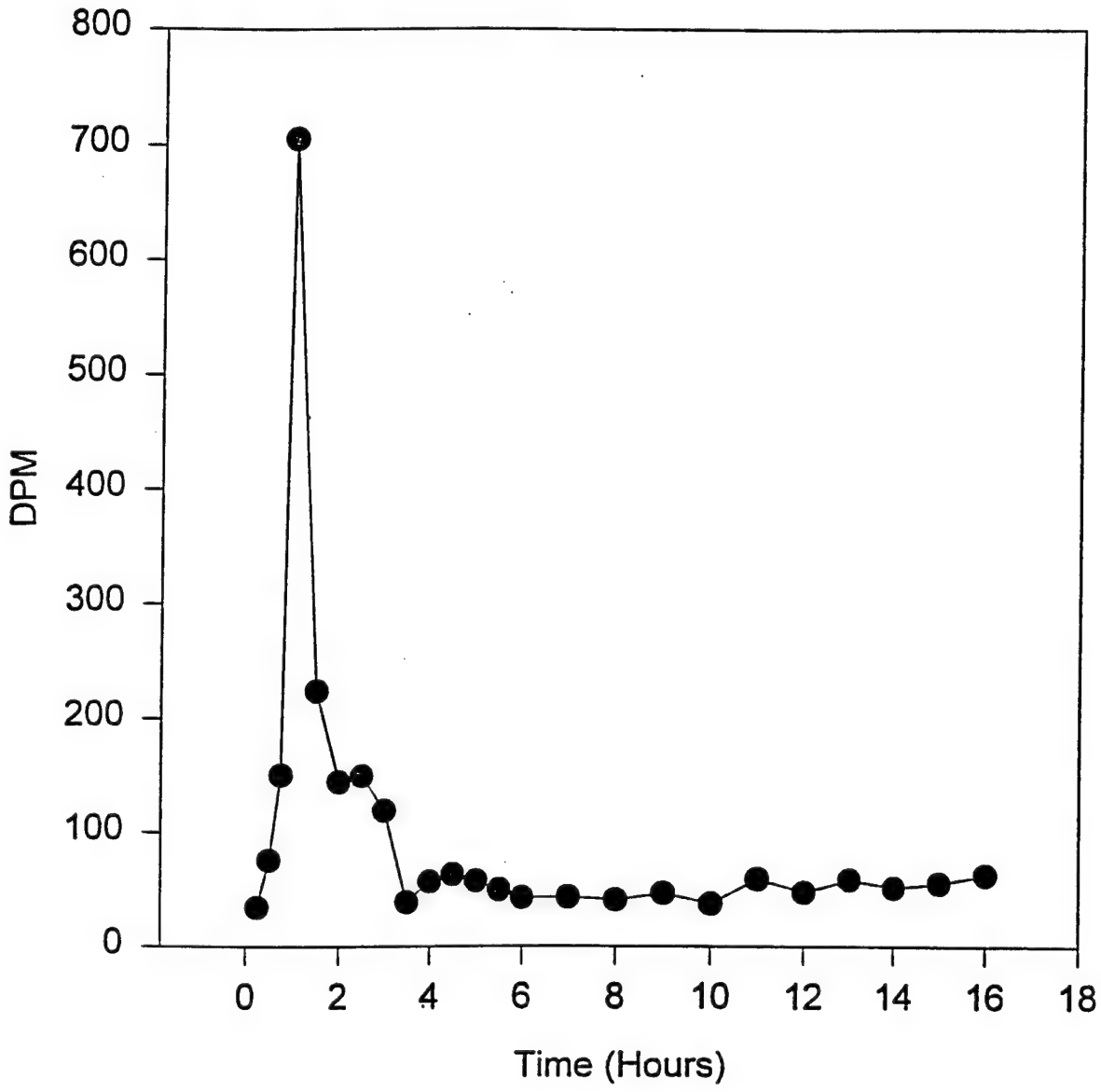


Figure 10.

Clearance of DBNP following oral dosage



phase of clearance from the blood in the first 60 minutes is followed by a steady state. This steady state continues for a week after the single initial dose and constitutes 4-6 % of the administered dose in the blood. The rapid phase of clearance is due to its distribution in all the tissues and its excretion through the urine and feces (Fig. 11a and b). Twenty four hours after a single i.p. dose of DBNP (0.4 mg/kg), 20% +/- 3.8 SD is excreted in the urine and 12% +/- 2.5 SD is excreted in the feces. The tissue distribution of DBNP 24 hours after a single dose (0.4 mg/kg) was: liver 14-16 %; spleen 3-5 %; kidney 8-10 %; heart 2-5 %; brain 0.8-1.2 %; muscle 0.5-1 %; fat 11-13 %; and blood 6-8%.

DBNP is excreted slowly from the body (Fig. 12). After a single dose of DBNP (0.4 mg/Kg), 82 -90% excreted in the urine and feces within 10 days. The excretion in the first two days averages about 18-20% for urine and 12-15% for the feces. The excretion rate in the urine and feces drop considerably from the third day (a 40% drop as compared to first two days) and continues to decrease slowly thereafter.

Urinary excretion of DBNP is lowered by 30 % in rats treated with 20 mg of Neomycin orally followed by a single oral dose of DBNP.(Vesselinovitch et.al. 1961) This suggests that intestinal microflora may contribute to the absorption of DBNP through the gastrointestinal tract after metabolic alteration by the microflora. Liver perfusion experiments clearly show that 4-5 % of the perfused amount is excreted in the bile.(Holder *et.al.* 1971)

The elution profile of the parent compound DBNP form C-18 reverse phase HPLC column detected at 450 nm is shown in Fig. 13. The retention time for DBNP is 37.55 min. under the eluting conditions described under the Materials and Methods Section.

Using identical conditions, the DBNP standard curve was generated (Fig. 14) by plotting the area under the curve against various concentration of DBNP. This standard curve is utilized to quantitate DBNP from biological samples.

The elution profile of the ^{14}C-DBNP under the identical eluting conditions is shown in Fig. 15. The difference of 2 minutes in the retention time between cold and hot DBNP is due to a delay in the starting time of the radioisotope detector after the injection plus the length of tubing between the column to the flow cell in the radioisotope detector.

Figure 11A.

Amount of radioactive label present in tissues 24 hrs post-exposure to DBNP

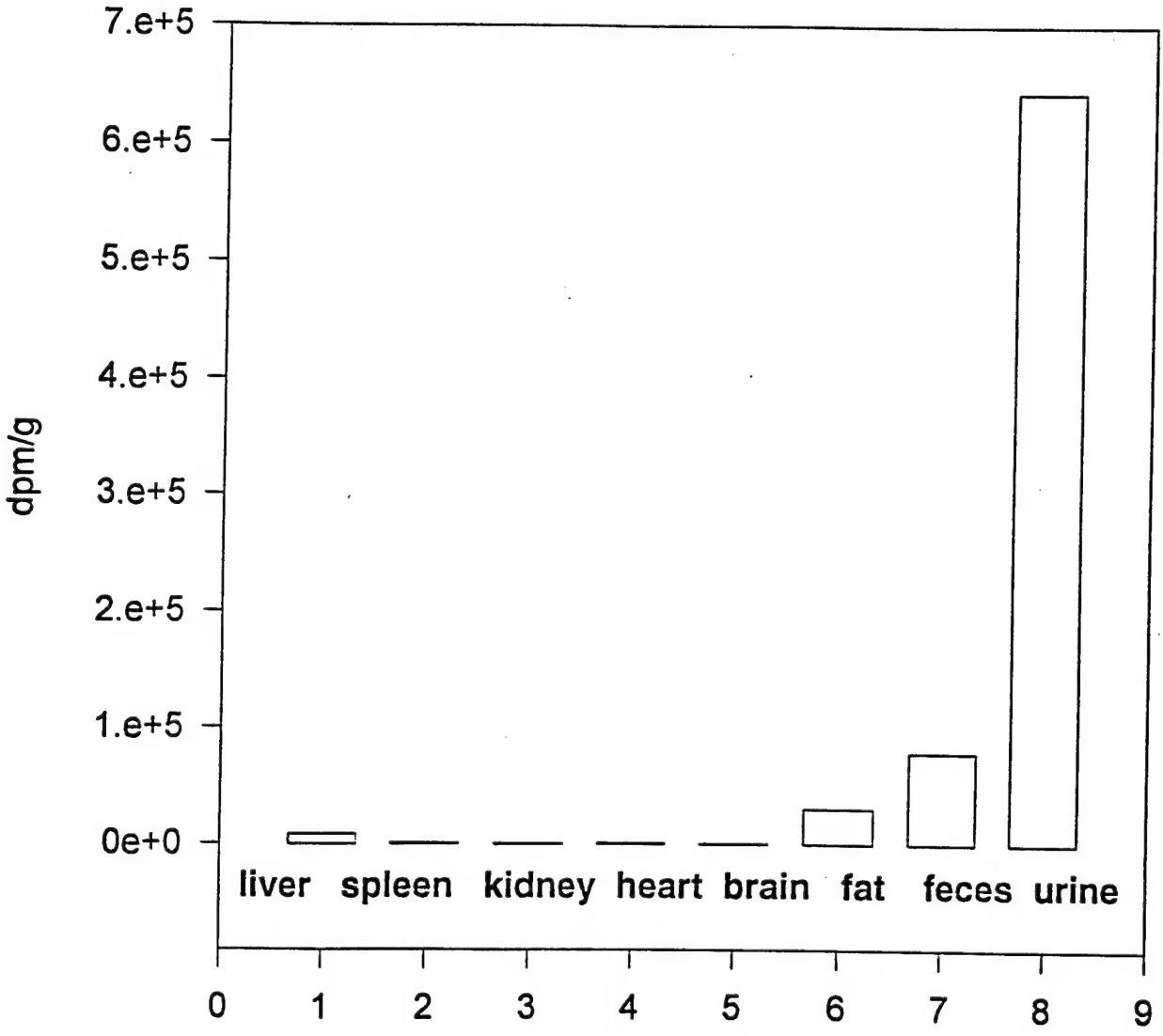


Figure 11B.

Distribution of DBNP in the tissues after i.p. injection

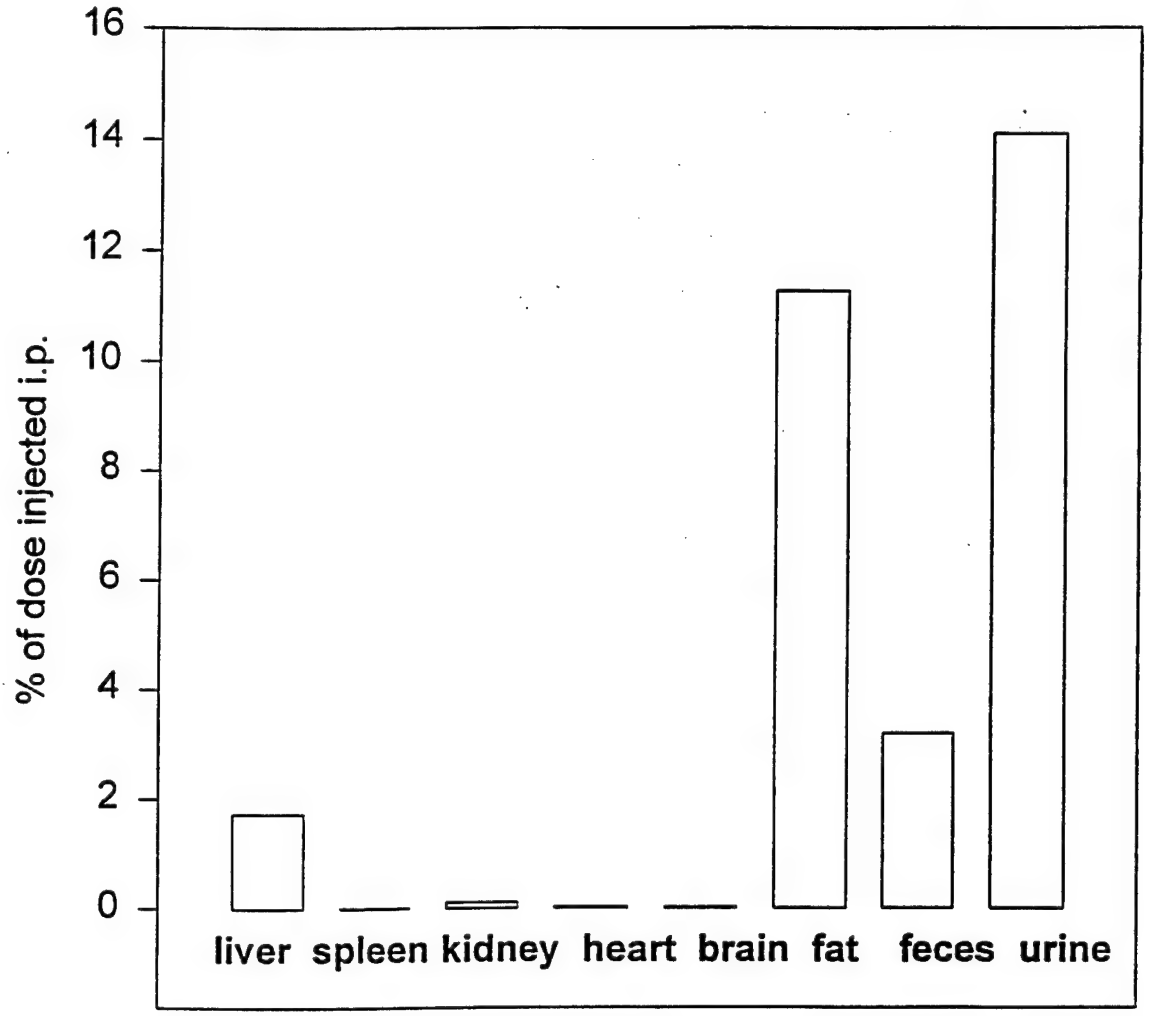


Figure 12.

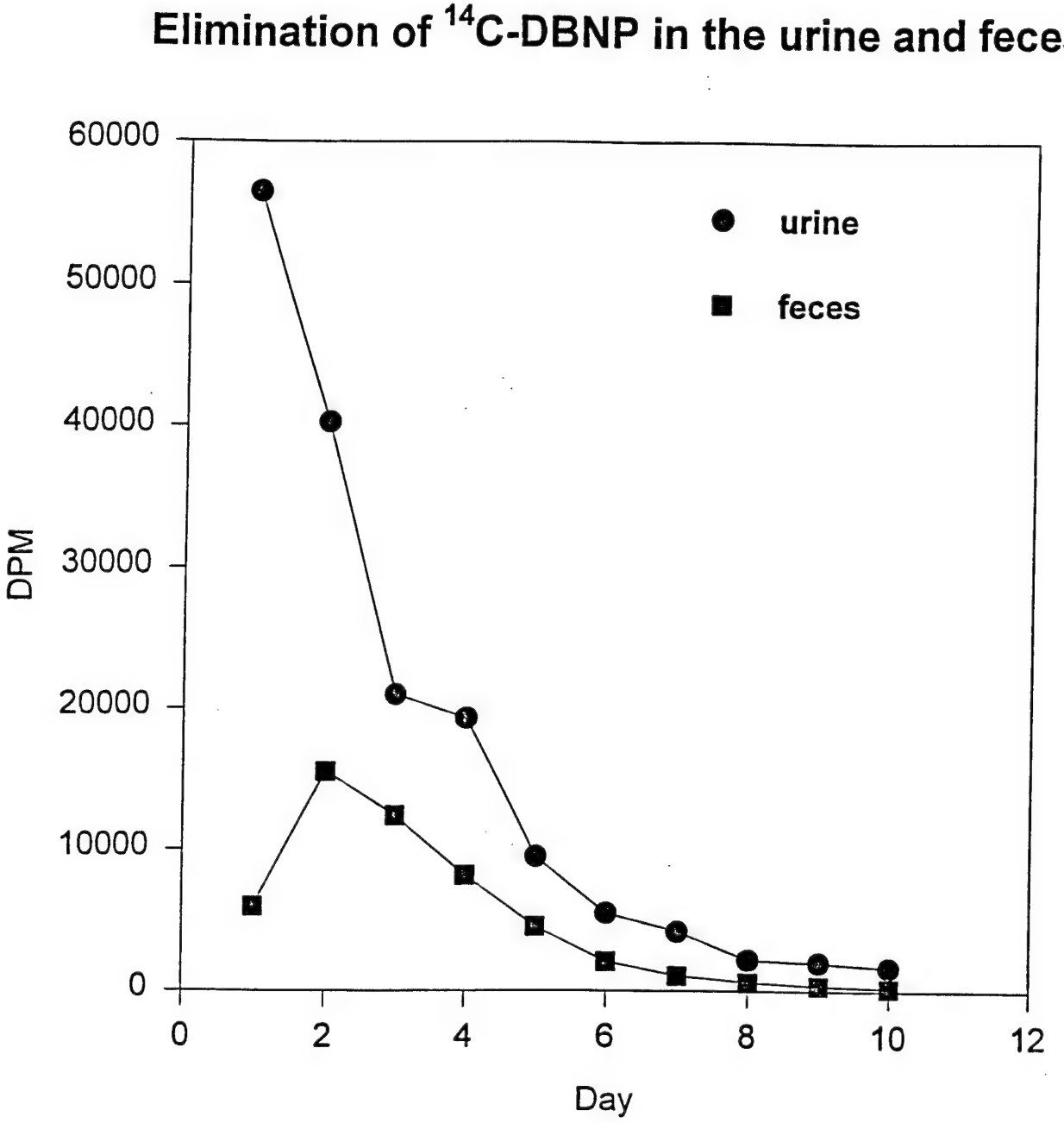

Elimination of ^{14}C-DBNP in the urine and feces
60000
50000
40000
30000
20000
10000
0
DPM
urine
feces
0
2
4
6
8
10
12
Day

Figure 13

Elution Pattern of DBNP from HPLC Chromatography

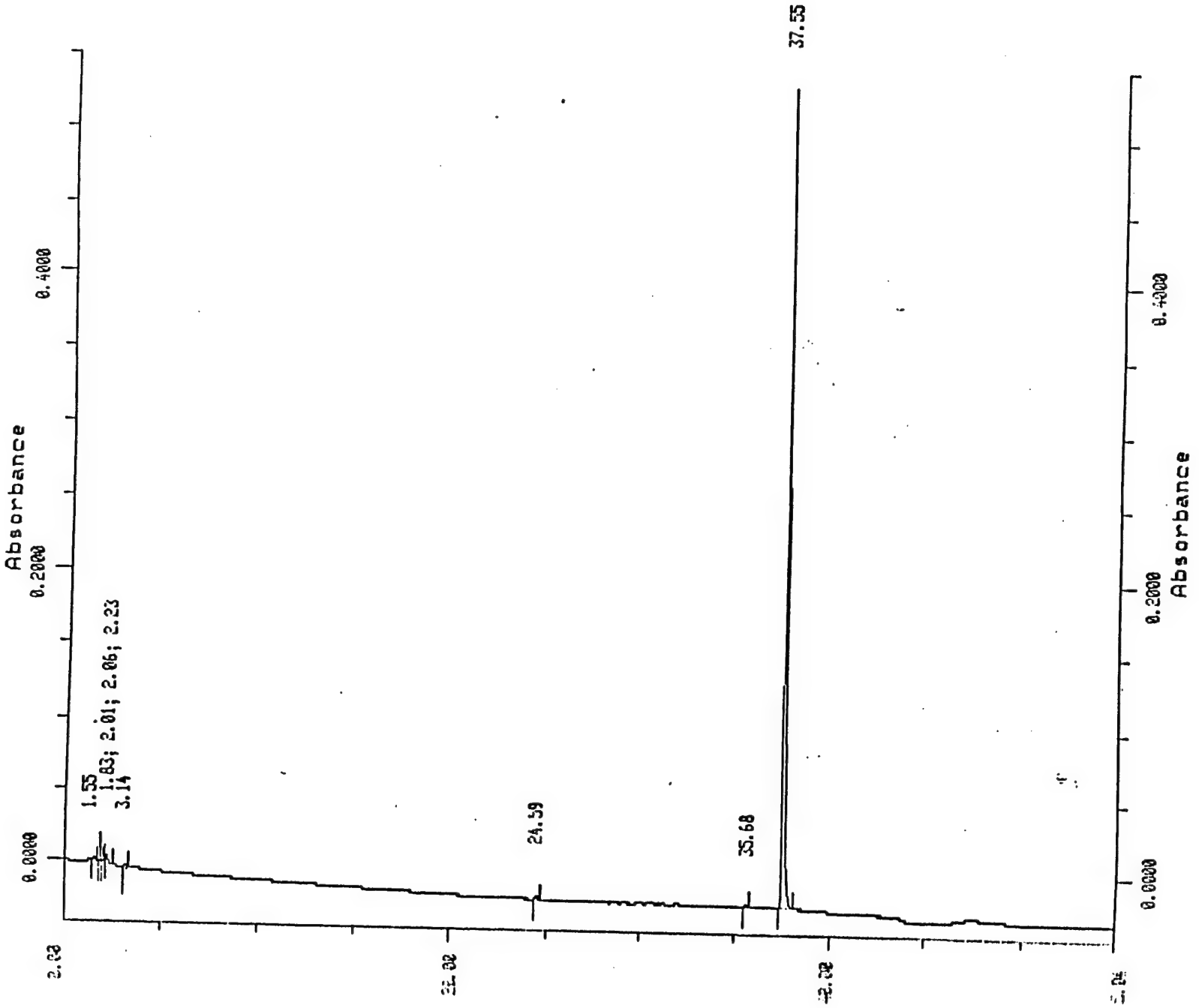


Figure 14.

Standard curve of the area of the DBNP peak via HPLC.

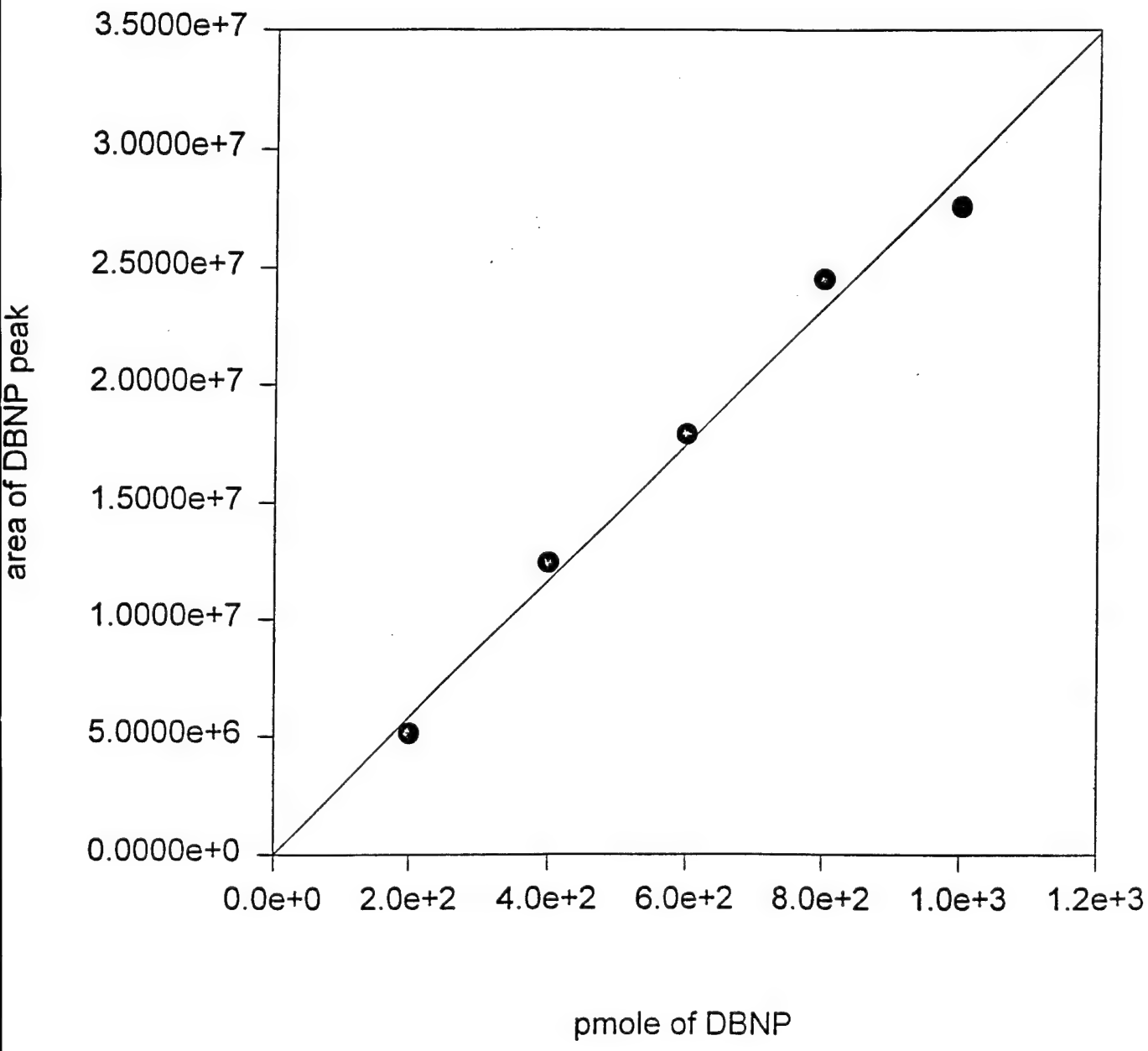
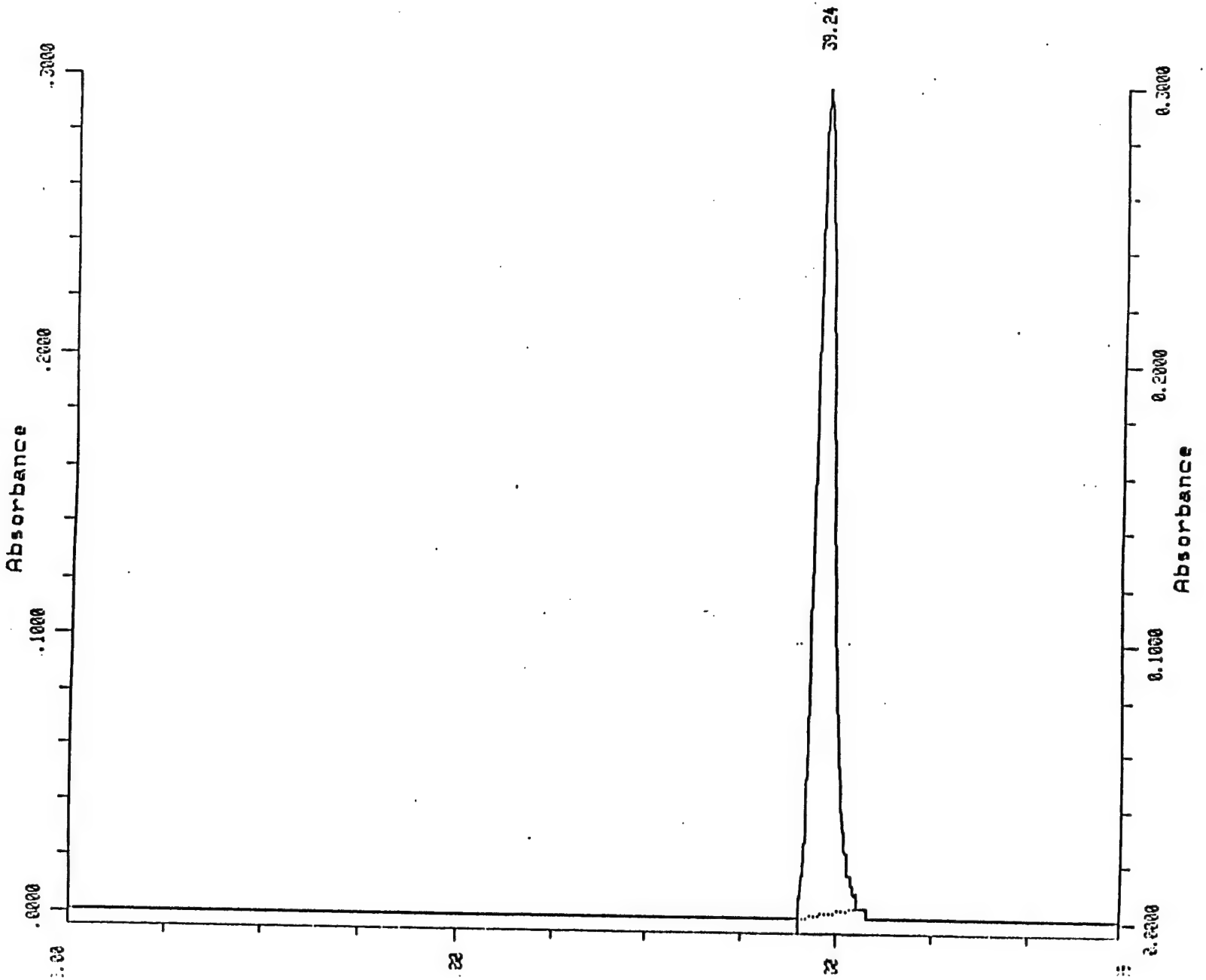


Figure 15

Elution Pattern of ^{14}C-DBNP From HPLC Chromatography



The elution profile of the metabolite isolated from the urine and feces is shown in Figs. 16 and 17. The metabolite was eluted earlier (25 minutes) than the parent compound DBNP (37.55). No parent compound DBNP are any other metabolite was detected in the purified samples from the urine and feces. The metabolite isolated from bile also eluted as a single peak in HPLC with the same retention time as the urine and feces sample.

The purified material from the urine, feces and bile was identified as a glucuronide conjugate after the radioactive material was digested with glucuronidase. The glucuronide conjugate DBNP is of the ether type. Generally the ether glucuronides are unstable in acidic conditions. After acid hydrolysis under nitrogen, the liberated glucuronic acid was isolated and crystallized. The presence of glucuronic acid was further confirmed by its melting point, reduction of alkaline cooper sulfate and naphthoresorcinol color reaction. Failure to identify unchanged DBNP in the urine and in the bile indicated that, once DBNP is aborbed, it is excreted after phase-II metabolism to a glucuronide conjugate. Acid hydrolysis of the metabolite followed by HPLC failed to show the presence of any other metabolite in which the nitro group is reduced.

Toxicity of DBNP at varied concentrations on rat and human liver slices is shown in Figs. 18 and 19. Comparison of nitrophenol toxicity in rat and human liver slices is shown in Table 2. Rat liver slices are more sensitive to DBNP insult than human liver slices. The order of susceptibility of both species was ATP content > protein synthesis > LDH release > K^+ leakage.

At a 50μM concentration, DBNP produced a 70% reduction in ATP content in rat liver slices as compared to a 30% reduction in human liver slices. At the same concentration, protein synthesis was reduced by 60% in rat liver slices as compared to 30% in human liver slices. At higher concentrations (200 μM), the toxic effects of DBNP are seen within an hour; whereas, at lower concentrations (25 μM), the toxic effect is seen after 4 hours.

Hepatocyte cells grown in tissue culture medium lose thier viability (100%) when exposed to DBNP at 2μg / ml concentration for 24 hours. Cells exposed to DBNP at 0.1 and 0.2μg /ml concentrations for 24 hours show a viability loss of 8% and 15%, respectively.

The effect of DBNP on state-3 and state-4 mitochondrial respiration are shown in Figs. 20 and

Figure 16. DBNP metabolite from urine

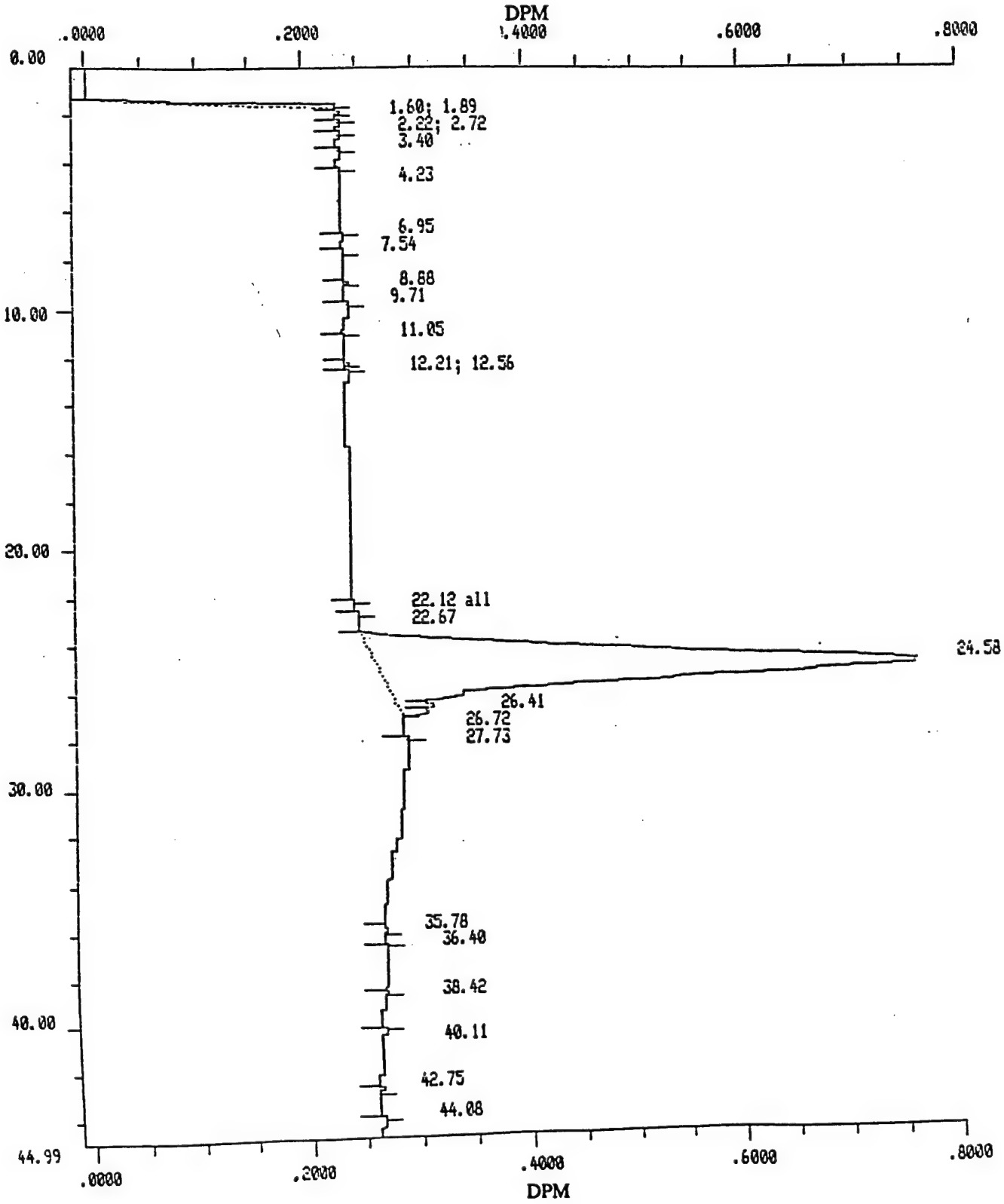


Figure 17. DBNP metabolite from feces

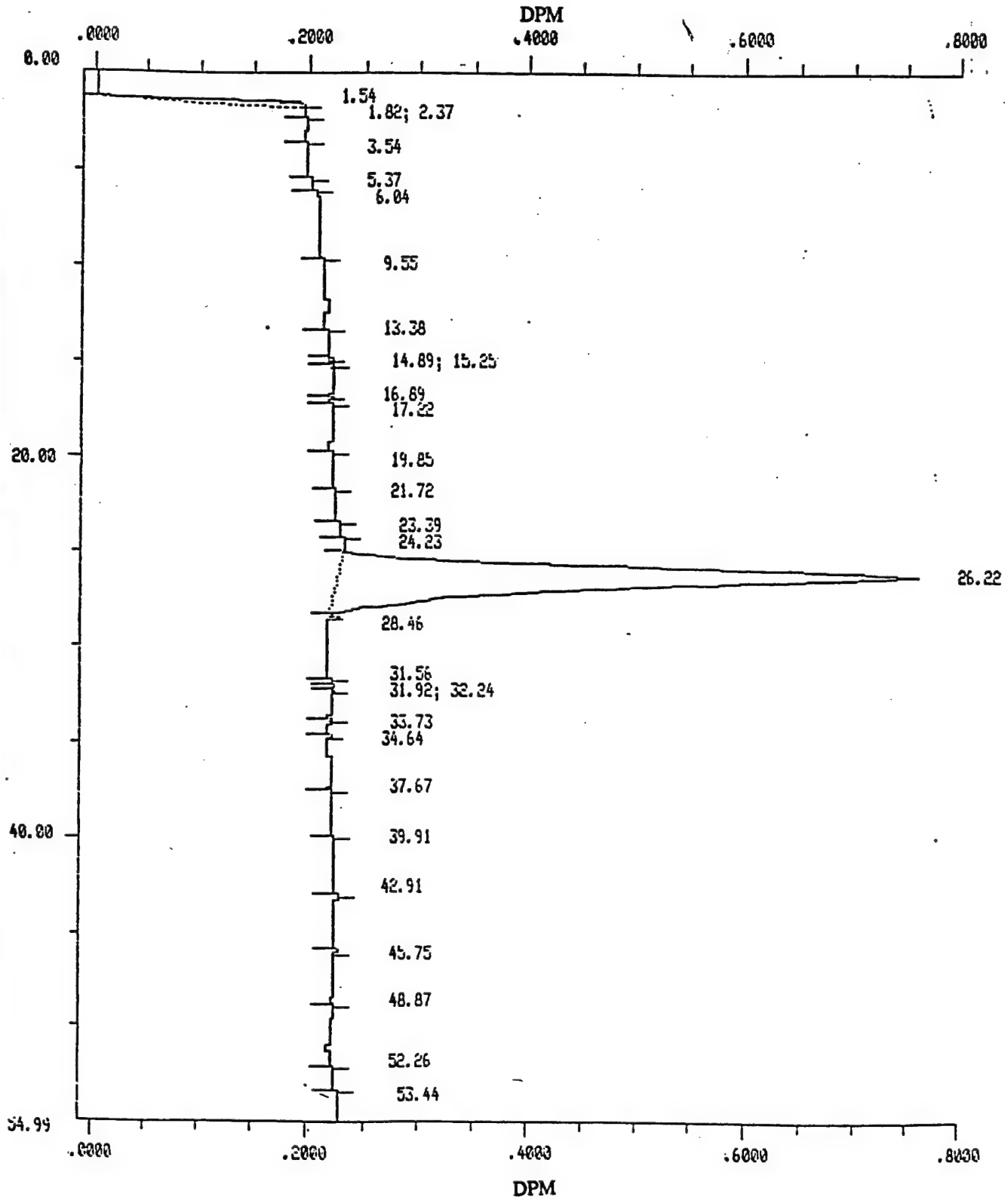


Figure 18. Toxicity of Varied concentrations of DBNP to Rat Liver Slices after Two Hours Incubation

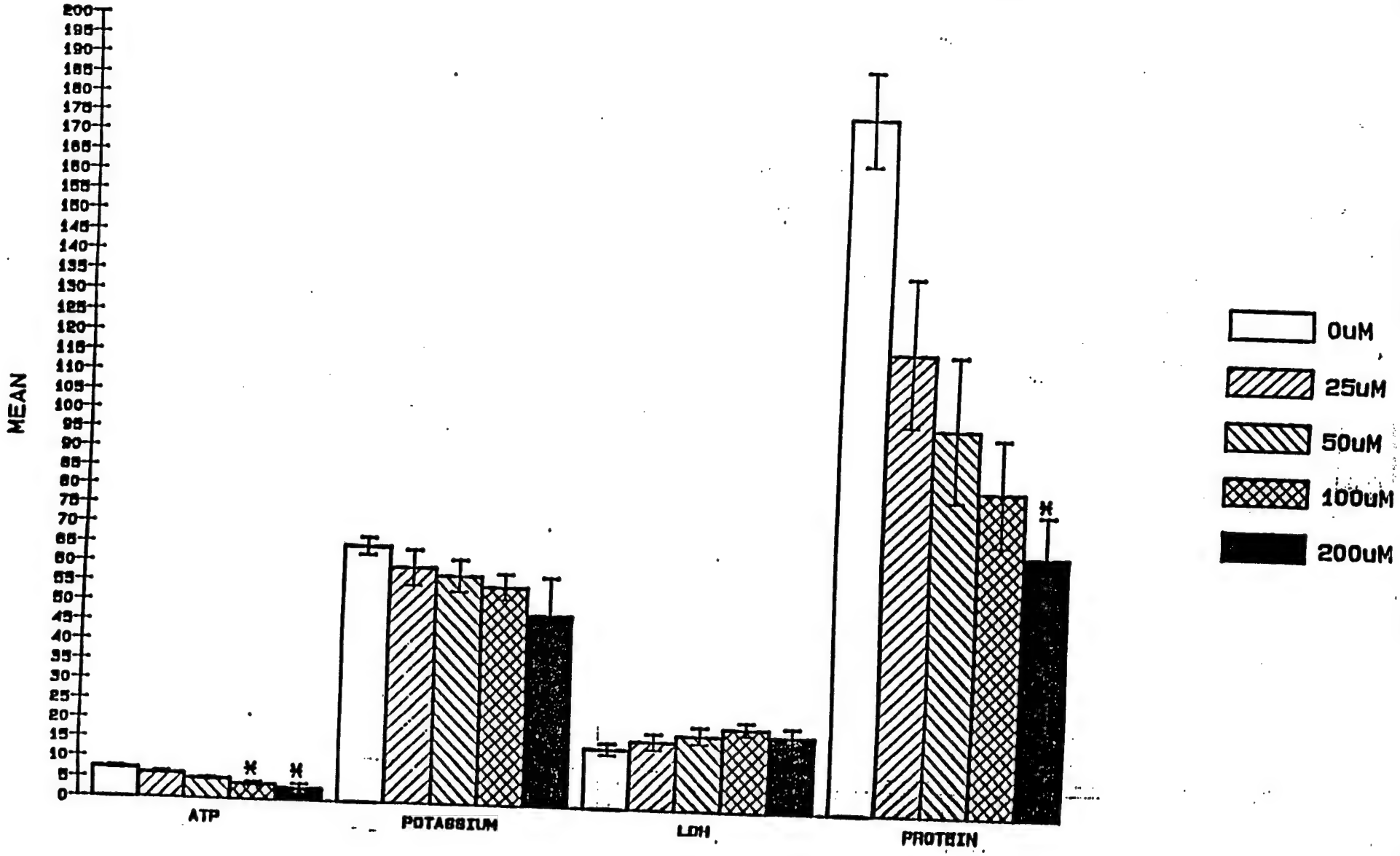


Figure 19. Toxicity of Varied Concentrations of DBNP to Human Liver Slices after Two Hours Incubation

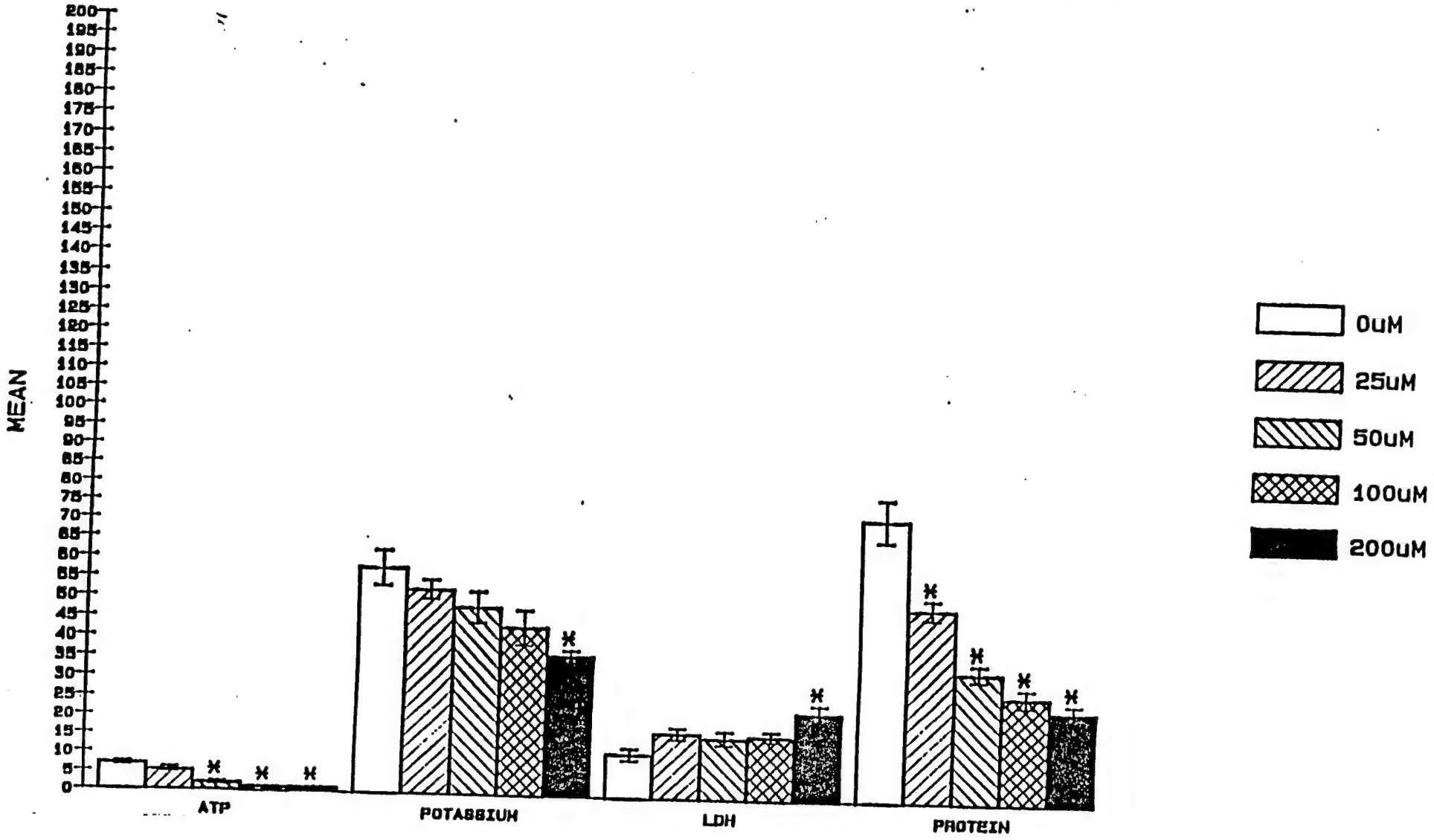


TABLE 2. Comparison of Nitrophenol Toxicity in Rat and Human Liver Slices.

	RAT		HUMAN	
Nitrophenol (50 uM)	ATP Content[a]	Protein Synthesis[b]	ATP Content[a]	Protein Synthesis[b]
Control	5.9 ± 0.7[c]	112.7 ± 23.2[c]	7.2 ± 0.4	177.3 ± 21.1
DN-*t*-BP	2.2 ± 0.0*	57.0 ± 19.3*	3.8 ± 1.7*	77.0 ± 12.2
Control	7.0 ± 0.7	73.0 ± 9.6	----------	----------
DBNP	2.2 ± 0.7*	34.3 ± 3.5*	5.1 ± 0.4	99.0 ± 32.7
2,4-DNP	6.1 ± 0.2	57.3 ± 28.0	6.5 ± 0.8	156.3 ± 26.8
4-NP	7.0 ± 0.6	79.0 ± 16.5	7.3 ± 1.2	142.7 ± 19.4

Values represent the mean ± S.D. of three determinations. The concentration and exposure time selected for comparison were 50 uM and 2 Hr, respectively.

a) nmo/mg wet wt.

b) DPM/mg wet wt.

c) Control values for DN-*t*-BP exposures were different from the control used for the other nitrophenols evaluated because these studies were conducted at a different time.

* Statistically different from control values; $p \geq 0.05$.

21. Both DBNP and DNP (a potent uncoupler of oxidative phosphorylation) inhibited mitochondrial respiration and ATP production. DBNP is one-third less potent than DNP inhibiting ATP synthesis in mitochondrial preparations.

DBNP (200 μ M) has no effect on FABP and sulfotrasferases in vitro.(Appendix-3, results, page-9). A slight increase in FABP was seen in DBNP perfused liver as compared to normal. (Table-1/ Appendix-c). No change in BST level was seen in DBNP perfused rat liver. (Table-2/ Appendix-c). In sub-accute toxicity studies the survived rats after 30 days (25mg/kg daily/i.p) showed decrease in body weight, increase in liver weight and increase in liver weight/body weight ratio.(table-3 / Appendix-c). Signifant changes were seen in the level OF FABP, DBT and BST in DBNP treated rats.

Figure 20.

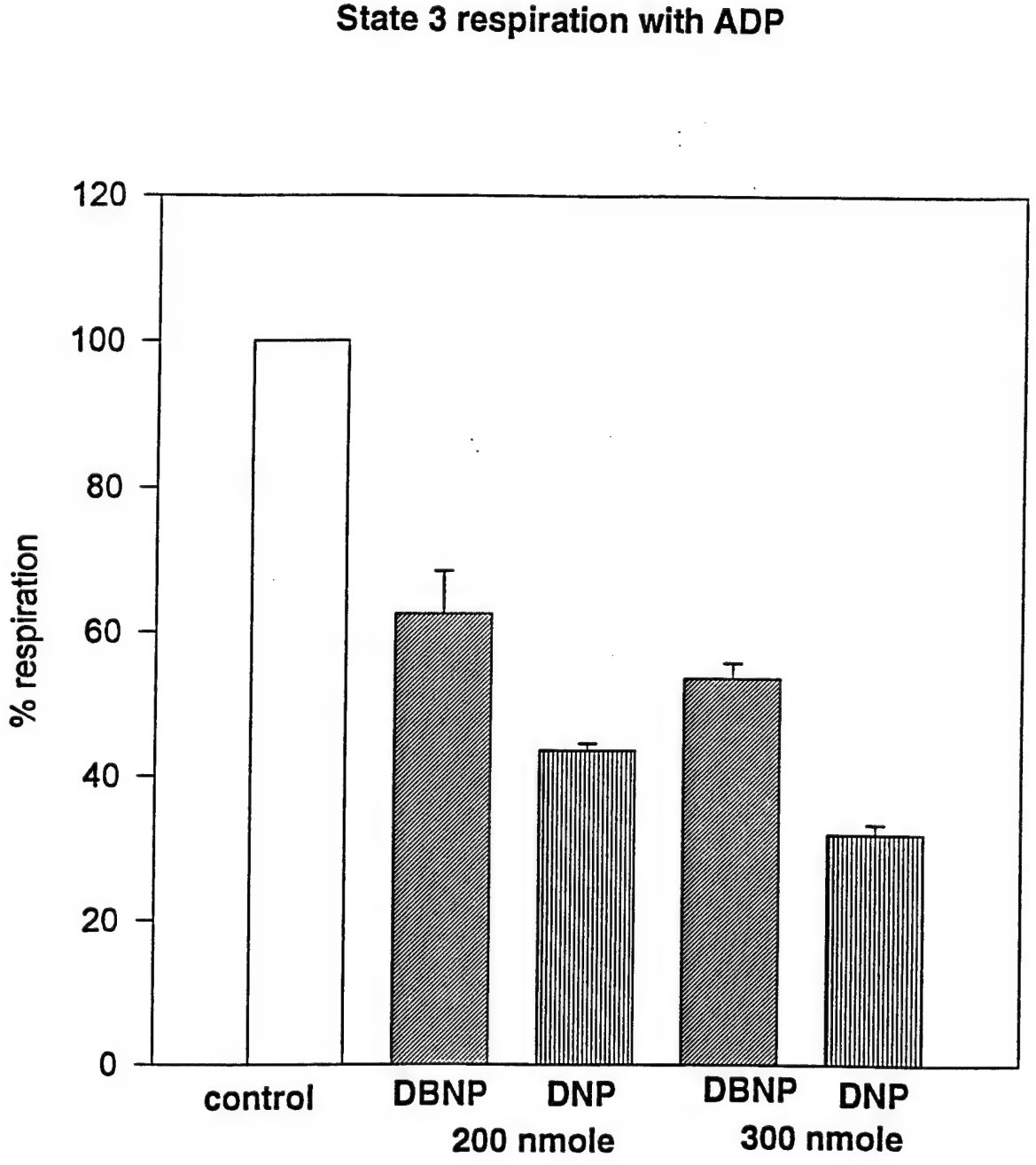
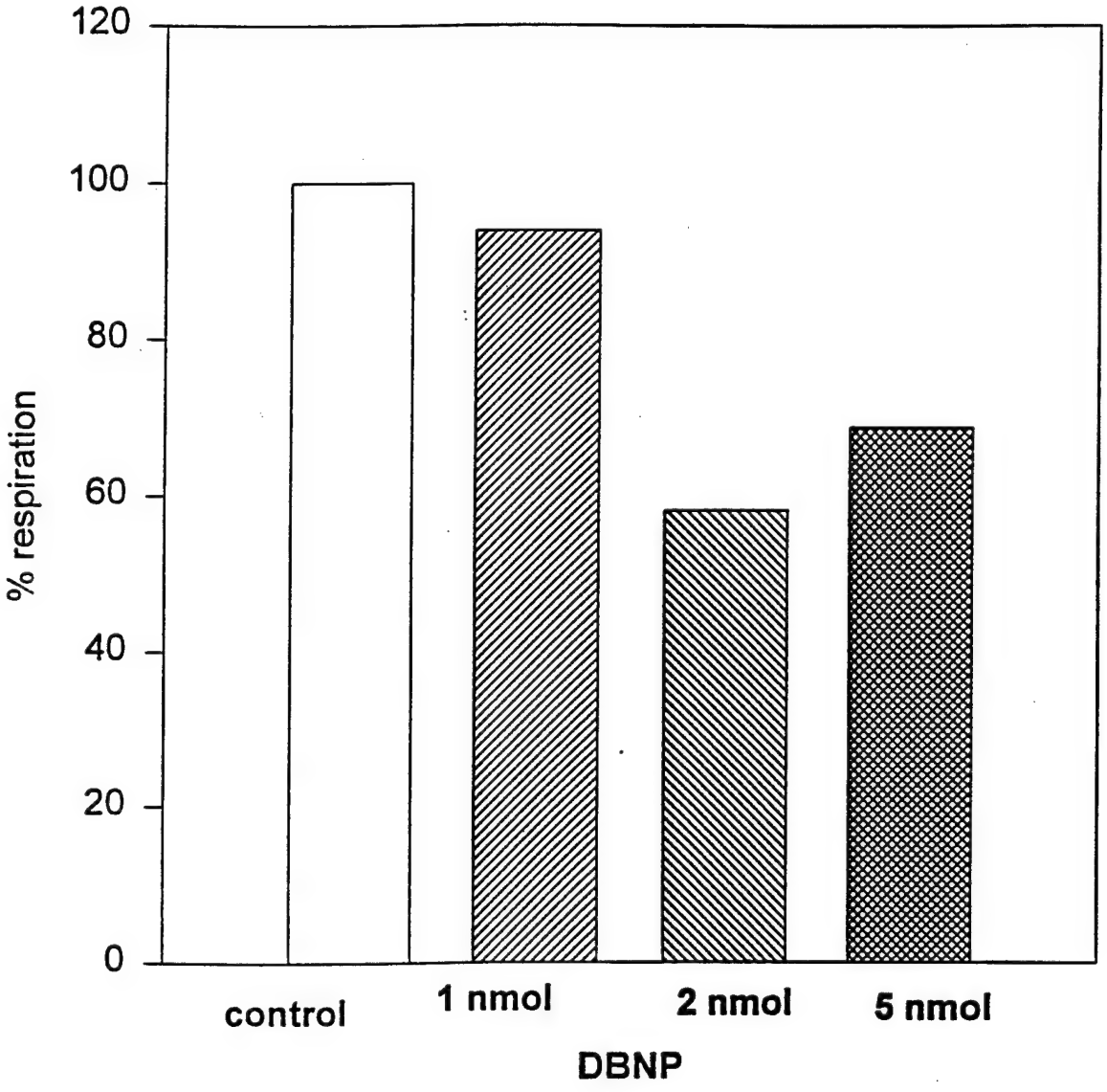

State 3 respiration with ADP
120
100
80
60
40
20
0
% respiration
control
DBNP
DNP
DBNP
DNP
200 nmole
300 nmole

Figure 21.

State 4 respiration with Succinate



SECTION 4

CONCLUSIONS

Combining literature information with the studies conducted under this work unit, one can draw the following conclusions concerning DBNP toxicity. The LD-50 dose by intraperitoneal injection in rodents is above 250 mg/kg, indicating that DBNP is of low toxicity. The oral dose was half as toxic as intraperitoneal administration in rats and guinea pigs. There is no sex difference, but rats are more sensitive to DBNP than other animals. High doses of DBNP applied on the rat skin did not show any toxicity or evidence of skin irritation, which suggests that there is low risk in dermal exposure.

Even though these acute toxicity test demonstrated that DBNP has a low toxicity in rats, mice, and guinea pigs, DBNP has a considerable tendency to produce cumulative toxic effects when administered repeatedly at one-tenth of the LD-50 dose. These observations suggest a potential health risk in situations where personnel may be repeatedly be exposed to even low doses of DBNP. DBNP is cleared from the system very slowly, and repeated exposures may slow the excretion further. These factors, combined with the fact that a considerable amount of DBNP is present in the fat and that the excretion rate drops after the first few days, contribute to the observed cumulative toxicity. The drop in the excretion rate may also be due to slow mobilization from the fat giving rise to a reduced level in the general circulation. The drop in the excretion rate is not due to reduced kidney function because water consumption and urine output remained the same as control. It is very likely that enterohepatic circulation of DBNP and its metabolite (see below) contributes to the delayed elimination of DBNP from the system.

DBNP is excreted in the bile as a glucuronide conjugate but not as a sulfate conjugate. HPLC analysis showed the presence of a single metabolite from the urine, feces or bile. Acid hydrolysis failed to show the presence of any other metabolite in which the nitro group is removed or reduced.

At the cellular level, the toxicity expressed by DBNP is very likely due to its inhibitory effect on

ATP synthesis, a well-known action of nitrophenols in general that leads to the inhibition of the number of biochemical pathways (anabolic and catabolic), particularly energy- dependent enzymes in carbohydrate metabolism. These biochemical actions lead to the fatty patches seen in the liver at higher does. In-vitro experiments with human liver slices indicate that humans may be less sensitive to the toxic effects of DBNP than other species (within the parameters studied thus far). With both in vitro and perfused liver experiments, DBNP has no effect on sulfotransferases and FABP, although repeated i.p. injections of DBNP (25 mg/kg; 30 days) caused an increase in FABP and a decrease in BST in-vivo. Thus, while in-vitro exposure to DBNP affects these fatty acid transport and metabolic factor, one cannot rule out the possibility that the histopathalogical changes seen in the tissues at high doses of DBNP may be due to its effect on other biochemical processes in combination with the inhibition of ATP synthesis.

SECTION 5

PUBLICATIONS AND PRESENTATIONS

1. J.A.Rivera, J.F.Wyman, D.L.Von Minden, N.Lacy, M.L. Chabinic, A.V. Fratini and D.A. Macys. (1995) Facile synthesis and physical and spectral characterization of 2,6-di-t-butylnitrophenol (DBNP): A potentially powerful uncoupler of oxidative phosphorylation. *Environ. Toxicol.Chem.* 14: 251-256.

2 J.Wyman, N.Reo, J.Rivera, T.Moore, S. Prues, D.Lee, C. Goecke and C. Alva. (1994) Hepatotoxicity. of 2,6-di-t-butyl-4-nitrophenol to isolated-perfused rat liver. *The Toxicologist* 14: 681.

3. J.F. Wyman, R.Fisher, S.L.Prues, C.D. Fleming and K. Brendel (1995) Comparative toxicity of 2,6-di-t-butyl-4-nitrophenol and other nitrated phenols in human and rat hepatic tissue slices. *The Toxicologist* 15: 1532.

4. S.S. Singer, M.Cunningham, T.Jewett and J.Wyman.(1995) Subacute 2,6-di-t-butyl-4-nitrophenol (DBNP) effects on the rat liver fatty acid binding protein and bile salt/dopamine sulfotranferase. *The Toxicologist* 15: 1689.

5. Synthesis and spectral properties of 2,6-di-*t*-butyl nitrophenol (March 15, 1994). Presented at the *American Chemical Society* 207th meeting held at Navy Post-Graduate Medical School, Monterey, CA.

6. Comparative toxicity of 2,6-di-*t*-butyl-nitrophenol and other related phenols in human and rat hepatic tissue slices. (March 2, 1994) Presented at the *Thirty fifth Navy Occupational Health and Preventive Medicine (NOHMP) workshop.* Held at Navy Environmental Health Center, Norfolk VA.

7. Subacute 2,6-di-t-butyl-4-nitrophenol (DBNP) effects on the rat liver fatty acid binding protein and bile salt/dopamine sulfotransferases. (March 12, 1995) Presented at *Society of Toxicolody Annual Meeting* held at Baltimore, MD.

MANUSCRIPT SUBMITTED

1. Absorption, Distribution, Metabolism, and Excretion of 2,6-Di-t-butyl-4-nitrophenol in Fischer-344 Rats. T.K. Narayanan, A.E. Jung, S.L. Prues, R.L. Carpenter, and K.R. Still. Submitted to *Toxicology Letters*.

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SECTION 6

REFERENCES

1. Appendix A (attached)

2. Appendix B (attached)

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6. J.Wyman, N.Reo, J.Rivera, T.Moore, S. Prues, D.Lee, C. Goecke and C. Alva. **(1994)** Hepatotoxicity of 2,6-di-t-butyl-4-nitrophenol to isolated-perfused rat liver. *The Toxicologist* **14:** 681

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8. S.S. Singer, M.Cunningham, T.Jewett and J.Wyman.**(1995)** Subacute 2,6-di-t-butyl-4-nitrophenol (DBNP) effects on the rat liver fatty acid binding protein and bile salt/dopamine sulfotranferase. *The toxicologist* **15:** 1689

NRL EFFORTS ON THE SUBMARINE SURFACE YELLOWING PHENOMENON

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NRL Involvement

Introduced to the problem and asked to get involved (early May)

Draw upon extensive submarine atmosphere (and materials) a lysis experience, as well as analytical resources

Learned of the knowledge to date, especially the important, initi work of EB

Mobil oil contains 2,6-di-t-butyl-phenol (DBP) and 2,6-di-t-but 4-methylphenol (DBMP or BHT)

2,6-di-t-butyl-4-nitrophenol (DBNP) identified (CAS #728-40-)

Questions

1. What is causing the surface yellowing onboard some submar es?
2. Is the nitrophenol (DBNP) identified by EB responsible?
3. How is the DBNP formed, in what quantities and where?
4. What are the precursors and what levels are found airborne nd on surfaces?
5. What are some possible solutions to this problem?

Sampling

Rode USS Maryland (SSBN 738) (May 26/27)
Acquired air (closed boat), surface swipe and oil samples
Received surface scraping samples from SSN 724
Visited EB and USS Annapolis (SSN 760) (June 11/12)
Acquired air (open boat) and surface swipe samples
Plan to ride USS Annapolis (June 30)

Laboratory work

Analysis of submarine samples

Thermal desorption of Tenax air samples
Solvent (CH_2Cl_2) extraction of surface swipe samples, scrapings or oils
Gas chromatography/mass spectrometry (GC/MS) analysis

Other experiments:

Exposure of phenols to NO_2
Attempts to sample airborne nitrophenol
Quantitation of airborne phenols

Results of Submarine Air Sample Analyses

USS Maryland (SSBN 738) (closed boat samples)

All samples showed DBP and DBMP, with increasing concentrations in more aft locations; much more DBP aft
The concentrations of the phenols were estimated to be XXX ppm
No samples yielded any detectable amount of DBNP

USS Annapolis (SSN 760) (open boat samples)

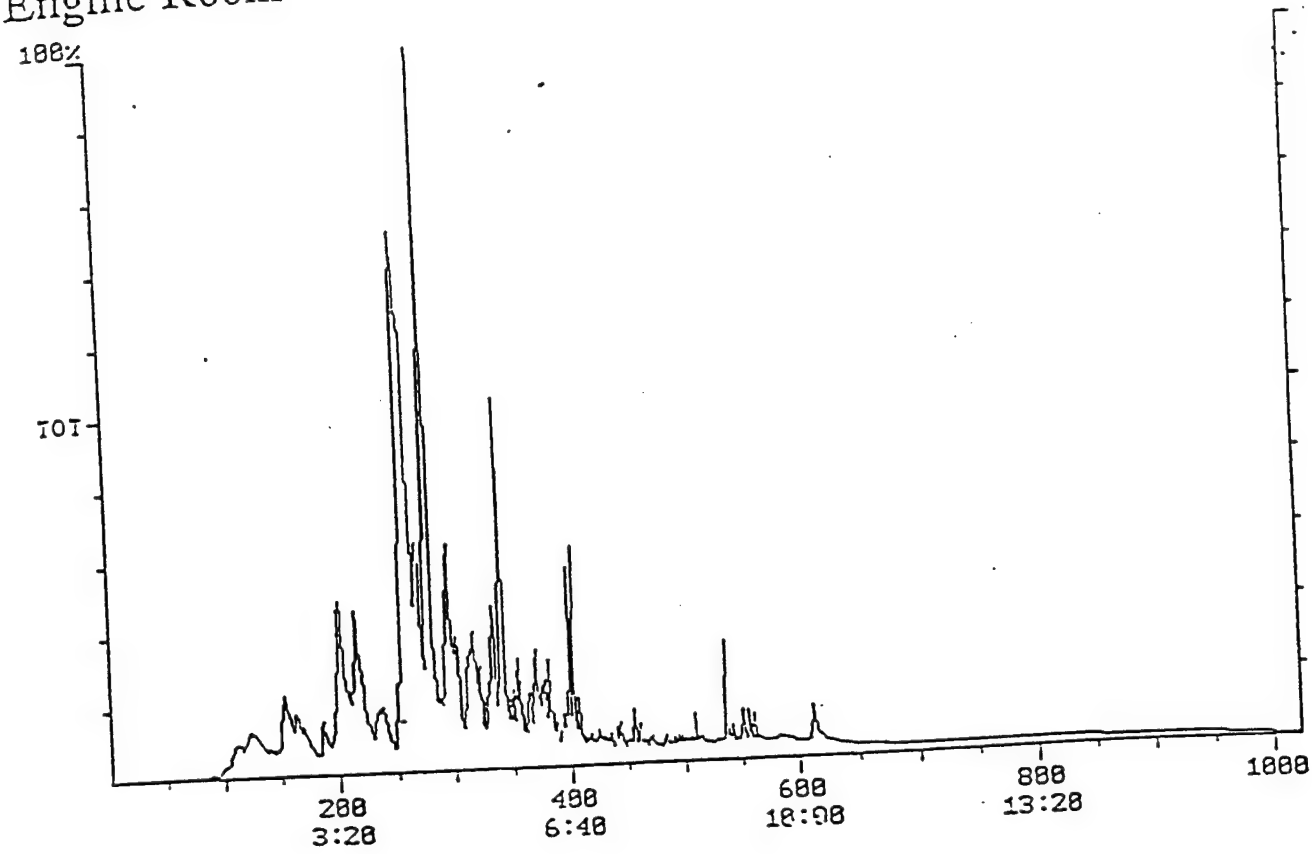
As on SSBN 738, DBMP but no DBP or DBNP were detected

Previous Submarine Air Sampling Trips - Not Targeting Phenols

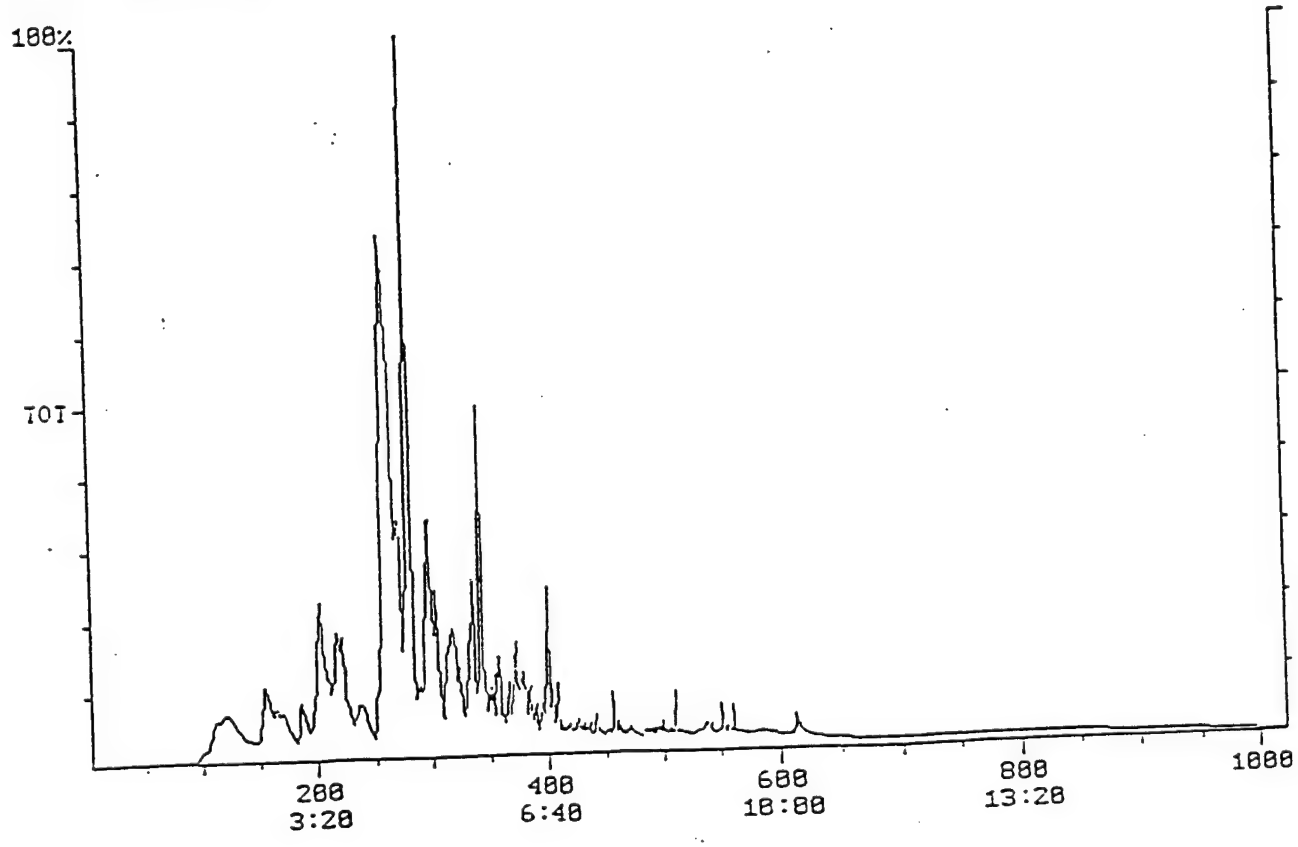
USS Pargo (SSN 650), USS Alabama (SSBN 731)

Airborne DBMP detected (background?) but no DBNP or DBP

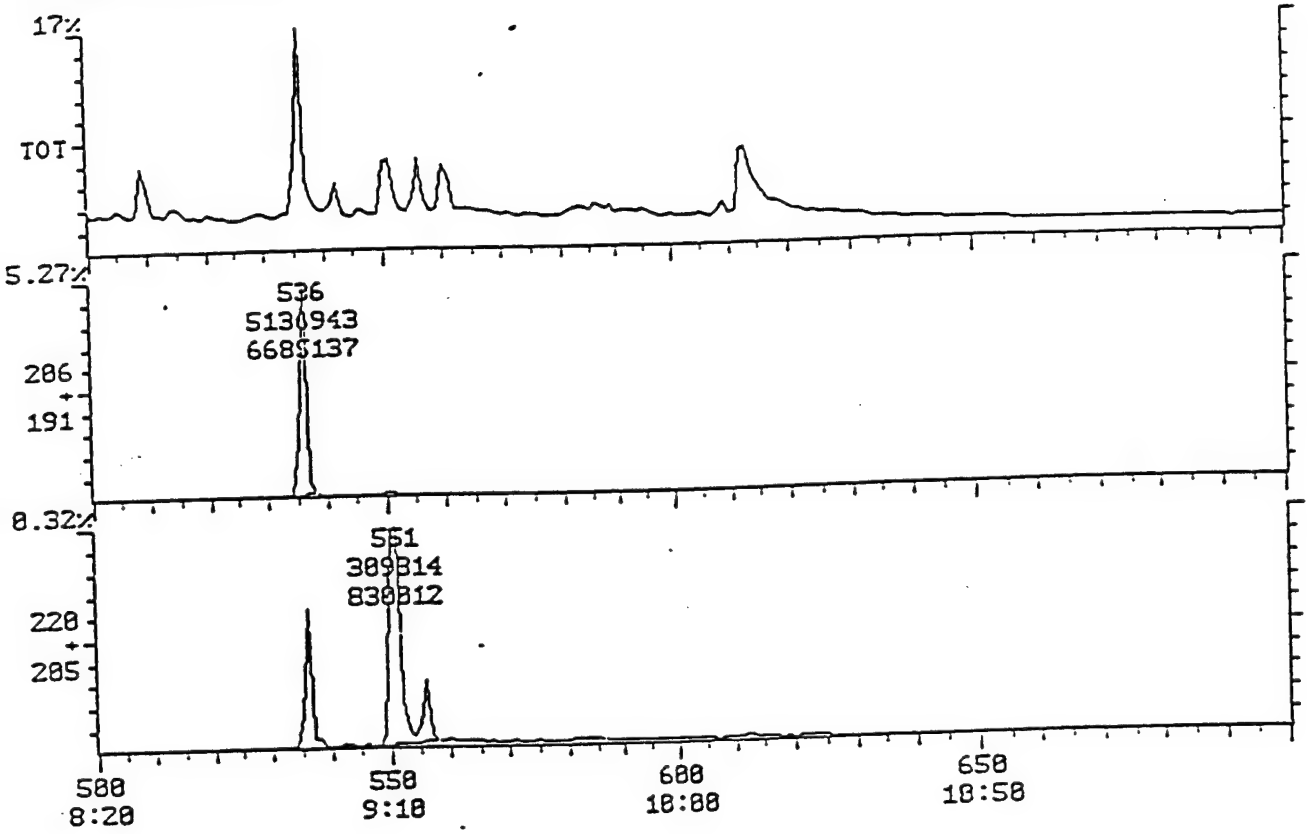
SSBN 738
Engine Room



SSBN 738
Near CAMS



SSBN 738
Engine Room



SSBN 738
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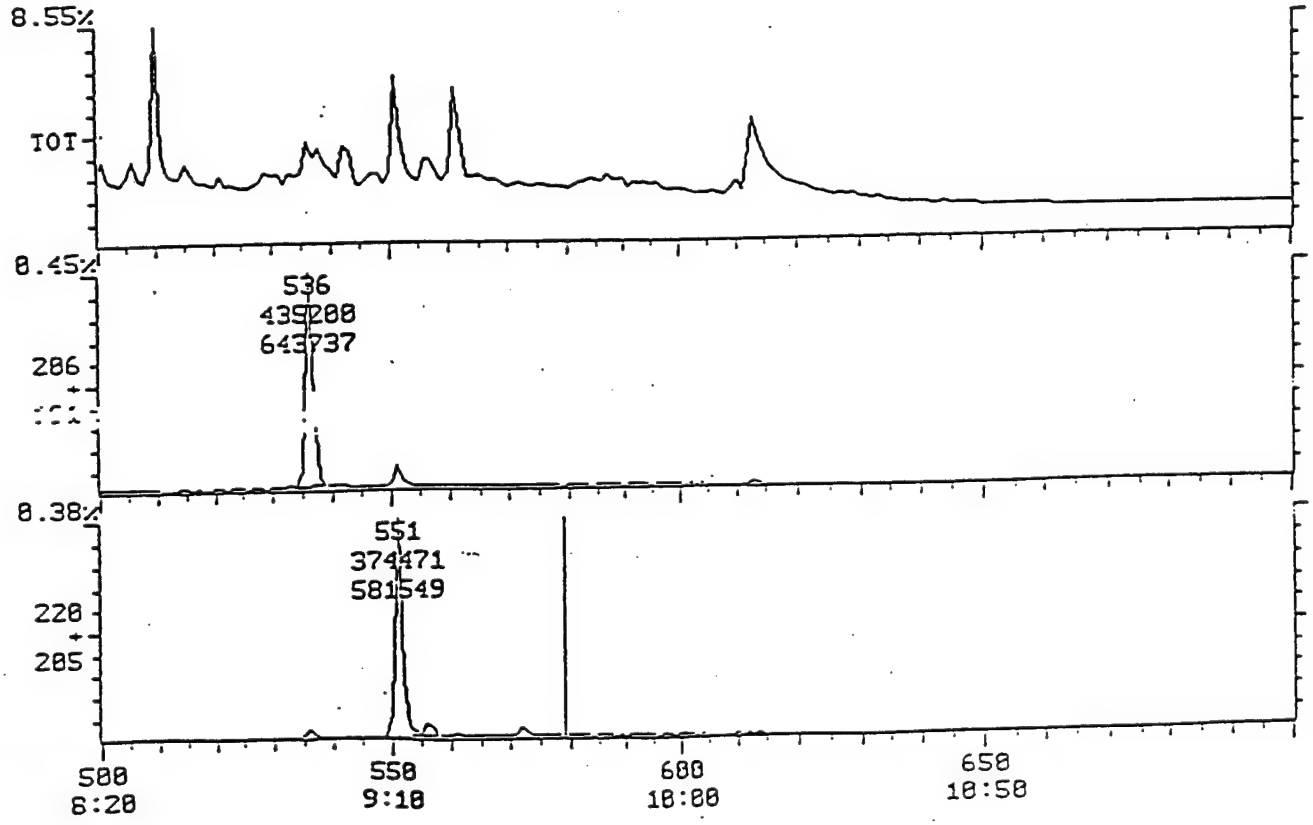

Results of Submarine Surface Swipe, Scrapings and Oil Sample Analyses

USS Maryland (SSBN 738)

Yellowed painted surfaces aft showed DBMP and DBNP, but little DBP

Non-yellowed surfaces did not show DBNP or DBMP (aerosols?)

DBP generally absent from surfaces, despite presence in air (reaction)

USS Annapolis (SSN 760)

Yellowed areas showed DBMP and DBNP, with low levels of DBP

Non-yellowed showed no detectable amounts of any phenol

Oil from the vent fog precipitator showed low level of DBNP

SSN 724

Visibly-discolored surface scrapings showed DBMP and DBNP, with no DBP

Results of Laboratory Experiments

Neither Tenax nor charcoal can sample DBNP when a solution of it is heated

Consistent with literature studies - vapor pressure must be very low

Impinger results

DBP and DBMP can be trapped in CH_2Cl_2, MEK

DBNP can be sampled with MEK only when the solution is heated to 100°C

DBNP can be formed by exposure of DBP to NO_2

10-50 ppm NO_2 + DBP yields DBNP in 1-3 hours

Exposed samples convert completely to DBNP overnight, with no NO_2

Nitration rate may be slightly increased with water

Oil samples exposed to 10-50 ppm NO_2 yield DBNP in 1-2 hours

Low level NO_2 exposure (sub ppm) results in slower reaction rates

Other Information

Literature references

German research on nitration of BHT and yellowing of textiles

Swiss work on attempts to sample phenols and nitrophenol from the air

DBNP: "... known to be the cause for various materials turning yellow in the indoor environment ..." - Rothweiler et al., Atmos. Envir., 25, 231 (1991)

LCDR Doug White's comments

Phenols are easily nitrated and, in general, nitrophenols are toxic, absorbed through the skin

Summary

- Significant amounts of DBP and DBMP are airborne (volatilized from the oil)
- DBP, DBMP and DBNP are present in swipes of yellowed surface areas
- It is unlikely that DBNP is airborne
- It is more likely that DBNP is formed by nitration of surface sorbed phenols

Unanswered Questions/Plans

- Continue sampling onboard submarines with and without the following problem
  - What are the sources and levels of NO_2, HNO_3 (and O_3?) in the air?
  - Do aerosols, temperature, humidity or light play a role in nitration?
  - Are there other compounds or reactions leading to surface discoloration?

Appendix B

STUDY OF VITRO AND IN VIVO EFFECTS OF DBNP ON RAT LIVER FATTY ACID BINDING PROTEIN AND RAT LIVER SULFOTRANSFERASES

Final Report for Contract F3360194MT601

Submitted, October 11, 1994

by

Sanford S. Singer Ph.D S. S. Singer

to

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I. INTRODUCTION

A. Initial observations on DBNP:

In 1992 the Navy Environmental Health Center, Norfolk, VA was made aware of concern about discoloration of bulkheads, bedding, and Naval personnel aboard submarines based at Groton CT. The agent which caused the problem was identified as 2,6-di-t-butyl-4-nitrophenol (DBNP). The yellowing process due to DBNP appeared to arise from reaction of 2,6-di-t-butylphenol, an antioxidant in lubricant 2190 TEP, with NO_2 produced by the action of their on board electrostatic precipitators. Exposure of submariners to DBNP and any potential toxicant action it might have appeared most likely to occur by the oral, inhalation, and dermal routes.

Exploration of the literature (1) showed that DBNP -- originally evaluated for use as a mitocide -- did exhibit substantial mammalian toxicity. This is evidenced by oral and intraperitoneal LD_{50} values for male rats, 270 and 450 mg/kg, respectively. Additional subacute toxicity studies (60 days, daily intraperitoneal injection) and chronic (16 week feeding) studies identified the cumulative DBNP toxicity in the liver, kidney, lung, heart, and spleen. Specific effects deemed pervasive included fatty liver, kidney dysfunction, and cardiovascular problems. A second study (2) produced much elevated liver weights and induction of Phase I and Phase II metabolic pathways. DBNP is also a potent uncoupler of oxidative phosphorylation (3).

It seemed of great interest to ascertain potential bases for toxicant action of DBNP in fatty liver, cardiovascular complications, and endocrinologic alterations. Likely candidates were

deemed to be the fatty acid binding protein of liver (FABP), bile acid, steroid hormone, and catecholamine/phenol sulfotransferases (STs). The first test parameter, FABP, was a likely candidate for examination due to its presumed importance in the biochemistry of lipids. STs were relevant because altering hormone/emulsifier forms via sulfation would affect endocrine responses of the Phase I/II metabolic enzymes. In addition, DBNP seemed likely to interact with FABP and STs due to its structural resemblence to the substrates and inhibitors of these enzyme proteins.

B. DBNP effects on the hepatic fatty acid binding protein:

The fatty acid binding protein (FABP) of rat liver is a 13-15 kdal protein that binds fatty acids and bile acids/salts (4-6). It is believed to be important to transport and metabolism of fatty acids and related lipids (7,8). Also, we have found that FABP can alter the sulfation of hepatotoxic bile (9) acids in vitro and that its tissue levels are altered by numerous lipophilic drugs and endocrine phenomena that disrupt or alter lipid metabolism (10-12). Very recently, its action in diabetes has been suggested (13). Hence, DNBP-tissue interactions could affect the in vivo levels of FABP, and by doing so such FABP alterations could induce fatty liver, cirrhosis and other types of liver disease.

C. DBNP interactions with sulfotransferases:

Sulfotransferase enzymes (STs) of liver catalyze addition of sulfate groups to many hydroxylated biomolecules, including the simple phenols, catecholamines, hormonal steroids, drugs, and

bile salts (see 14, and reviews, 15, 16). They utilize 3'-phosphoadenosine-5'-phosphosulfate (PAPS) as the sulfation coenzyme. Several observations, here, suggest the value of the exploration of DBNP as a sulfation substrate and/or inhibitor. First, p-nitrophenol is a substrate of phenol and catecholamine STs. Second, phenols with bulky substituents (e.g., the polyhalophenols) inhibit phenol STs . Bile acid sulfation will alter the emulsifier capacity of liver and could thus be important to the definition of the lipid content of liver.

II. RATIONALE AND AIMS

It is proposed that some aspects of DBNP action in fatty liver and the other toxicant effects observed may be related to alteration of the FABP and the bile salt/hormone sulfotransferase levels that can be shown by examining a male rat model system in animals from the Naval Medical Research Institute (NMRI) colony, These rats will be used to simplify future comparison with other data obtained by researchers at NMRI. Our aims will be to:

a) examine in vitro effects on FABP and rat liver sulfotransferases that catalyze sulfation of bile salts, catecholamines, and steroid hormones

b) compare effects on these proteins of DBNP perfusion of livers and perfusion with Ringer's solution

c) identify the results of subacute DBNP administration on the parameters identified in a and b.

III. EXPERIMENTAL METHODOLOGY

A. Animals and chemicals:

Male Fischer rats (Charles River, Boston, MA IN) were purchased

at 150-175 g, maintained under controlled conditions at the NMRI and used at 250-400g. All the rats were fed rat chow and water, ad libitum. Rose bengal, enzyme grade sucrose, Trizma (trishydroxymethylaminomethane), KCl, NaCl, and glycolitholithocholate were purchased from Sigma-Aldritch (St. Louis, MO), DEAE-Sephadex A-50, Sephadex G-25, and Sephadex G-75 came from Pharmacia-LKB (Piscataway, NJ), and [^{35}S]-3'-phosphoadenosine-5'-phosphosulfate (PAPS, 2.00 Ci/mmol) were from Dupont-New England Nuclear Inc. (Boston, MA). Unlabeled PAPS was prepared as we have described earlier (17). All of the other reagents and supplies used were of the finest quality available. from standard suppliers.

B. Cytosol, sulfotransferase and initial FABP preparation: Cytosol (18) was prepared after rats were decapitated and livers were quickly removed, trimmed, chilled, and homogenized in 1 mL g^{-1} of 1 C, 0.050 M tris-0.25 M sucrose-3.0 mM 2-mercaptoethanol, pH 7.5 (Buffer 1). Homogenates were centrifuged for 1 h (105,000 x g, Beckman L-565) at 1 C. Then, clear, supernatant cytosol was filtered through 0.2 micron filters and used as the source of FABP and all sulfotransferases (STs).

STs and FABP were in DEAE-Sephadex A-50 fractions. The ion exchange chromatography was performed with cytosol samples from 5 g liver, applied to 1.35 x 23 cm DEAE-Sephadex A-50 columns prepared as we have already described (18). Nearly identical ion exchange chromatograms on two size-paired columns were carried out simultaneously when cytosol samples from experimental and control rats were used. Each chromatogram was developed with a linear gradient that was made up of equal volumes of Buffer 1

and Buffer 1- 500 mM KCl (300 mL each). Liver samples were each applied to the columns nearly simultaneously. The chromatography was followed by examination of A_{280} nm absorbance (protein content) of effluent fractions. Then, measurement of FABP and ST content was carried out as described. Figure 1 shows the FABP and protein content of effluent fractions.

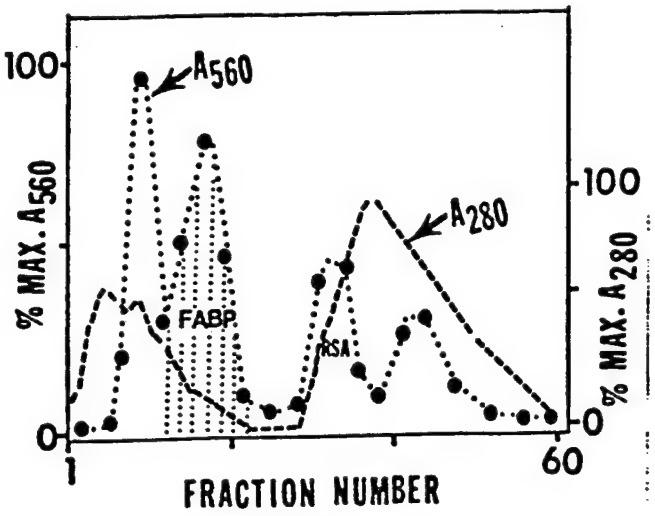

Figure 1 An A-50 chromatogram, showing protein and FABP content

C. FABP quantitation:

This measurement used the rose bengal assay we describe elsewhere (9). Test samples were mixed with ice cold Buffer 1 and 8.00 nmol rose bengal, to yield a volume of 0.700 mL, and incubated (5 min at 4° C). Each reaction mixture was used to quantitate FABP content by a Sephadex G-25 column method or by the equivalent dextran-coated charcoal assay. The two methods were in agreement within 5%. The absorptivity per mg FABP was 1.29.

D. Sulfotransferase assays:

BST assays were carried out by a method we report elsewhere (9, 14). The details are given as an example. Aliquots, 0-0.20 mL of enzyme were mixed with 0.55 ml of 10 μM bile salt in 100 mM KH_2PO_4 (pH 7.00). The sulfation reaction was started with 100 nmol [^{35}S] PAPS (2,000 dpm $nmol^{-1}$) and carried out for 30 min. The reaction was stopped by boiling for 1 min; reaction mixtures were cooled on ice; 2.00 mL of 1.0 M ammonium hydroxide and 5.00

mL of n-butanol were added and mixed for 30 sec. Then, aqueous and butanol layers were separated by centrifugation (5 min, 2000 x g). Samples of butanol layers were counted to identify enzyme levels. Assays of steroid, catecholamine, and phenol sulfotransferase activity we also designed are not described here. The descriptive data are found in our earlier papers (19-21).

E. Statistics:

The statistical significance of the differences between control and experimental groups was determined via Student's test(22).

F. Radioisotopic methods:

The sulfated reaction products were mixed with 5-7 ml of Riafluor scintillation cocktail (Dupont-NEN, Boston MA), depending on the assay method, and counted in an Intertechnique SL-30 scintillation counter, using the channels ratio method (23).

G. FABP isolation:

This process, which we described elsewhere (9), began with the DEAE-Sephadex chromatography cited in III.B. The effluent fractions described were assayed with rose bengal and those which contained FABP were pooled and concentrated to five mL in an Amicon vacuum concentrator, using YM5 membranes. Each concentrated FABP pool was then applied to a 1.95 x 70 cm Sephadex G-75 column, preequilibrated with and eluted with Buffer 1. Again, control and experimental samples were eluted simultaneously from paired columns of the same dimensions. The effluent fractions collected were then assayed for protein and FABP (e.g., Figure 2). The FABP-containing fractions -- highly purified FABP were then pooled and reassayed to identify rat liver FABP content in

mg per g liver or per 100 g bodyweight (BW).

IV. EXPERIMENTAL DESIGN

A. Study of effects of DBNP on hepatic FABP and the ST activity:

Here we will identify the interactions of DBNP with rat liver FABP and with phenol/bile acid/steroid STs, to quickly probe whether the toxicant has any inhibitor potential. Also the ability of DBNPas a phenol ST substrate will be explored.

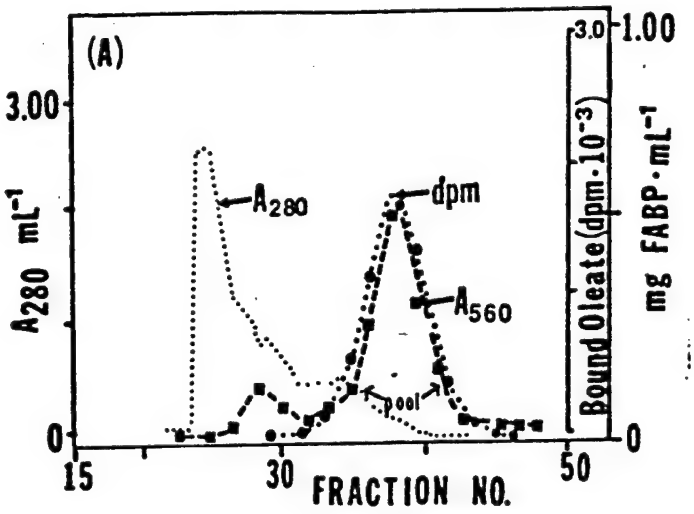

Figure 2 A Sephadex G-75 chromatogram Depicting FABP elution

B. Identify whether DBNP in perfusions alters FABP or ST levels:

This study involves examination of liver by cytosol assays and column chromatography described above (on DEAE Sephadex A-50, and Sephadex G-75 columns). The endeavor will quickly probe whether the toxicant has the potential to alter lipid homeostasis by changed FABP or ST levels. It utilize tissue from the standard perfusions in progress at NMRI (25). The perfusions will be carried out at NMRI. The livers will then be placed on ice and taken to my laboratory for immediate processing.

C. Understanding effects of DBNP subacute toxicity on FABP and targeted STs:

This may add a dimension to your ongoing endeavors by enabling preliminary identification of factors associated with fatty liver

and cardiovascular damage already observed (2) The experimental design will be as cited. Rats will be given vehicle or DBNP daily intraperitoneally. At chosen intervals they will be sacrificed and their livers will be perfused for 5 min to bring them to a state similar to that obtained in studies carried out at NMRI. Then, they will be placed on ice and taken to my laboratory for immediate processing.

V. RESULTS

A. In vitro DBNP effects on rat liver FABP, BST, DST, HCST, and EST

In these experiments study of cytosol and DEAE Sephadex A-50 column fractions indicated (3 experiments, not shown) that there were no meaningful differences in BST or DST activity in samples assayed with or without 200 μM DBNP. Similar examination of FABP in Sephadex G-75 column fractions or purified FABP pools from columns (3 experiments, not shown) did not uncover any effects of 200 μM DBNP on FABP activity.

We were unable to carry out a successful examination of DBNP effects on HCST or EST, as the cortisol and estrogen ST assays did not work under any experimental conditions (8 experiments, not shown). This implies that a component of perfusion mixtures used at NMRI prevents the reaction because we carried out successful training assays with untreated liver from rats from our own animal colony.

DBNP was not sulfated by cytosol or ion exchange chromatogram fractions (4 experiments, not shown).

B. Effects of perfusion of control livers with DBNP or Ringer's solution on FABP and STs

1. FABP levels per g liver and per 100 g BW were elevated by DBNP perfusion: Table 1 shows the FABP levels per g and data extrapolated to per 100 g BW in rat livers from males carried through the routine perfusion procedure used at NMRI, with or without 0.36 mM DBNP. Shown is one of two experiments that provided similar data. An average 25% increase of the FABP isolated from the livers perfused with DBNP was obtained.

Table 1 Effect of DBNP perfusion on FABP levels in livers of representative male rats

Exptl Gp	BW(g)	LW(g)	Hepatic FABP in mg FABP per: g liver	100g BW
Control	371	9.46	0.873	3.56
DBNP	372	12.1	1.07	4.34
% FABP Increase			22.6	21.9

2. BST levels per g liver and per 100 g BW were not elevated by DBNP perfusion: Table 2 gives the observed BST levels per g lover and data extrapolated to per 100 g BW in livers carried through the routine perfusion, with or without DBNP. Shown is one of two experiments that provided similar data. A small average difference of under 5% was observed. This was within the expected error of the assay procedure. No differences of individual BST isoenzymes were seen in DEAE Sephadex A-50 chromatography (not shown).

Table 2 Effect of DBNP perfusion on BST levels in livers of representative male rats

Exptl Gp	BW(g)	LW(g)	Hepatic BST in nmol GLCS per: g liver	100g BW
Control	272	9.85	249	904
DBNP	271	11.5	226	944
% BST Change			-9.24	+4.42

3. DST levels were not be obtained, as the cytosol assay was unsuccessful: The DST assay was not successful during the time period in which this experiment series was carried out. We later found that the problem had to do with the long perfusion time and the heparin content of the perfusate. Hence, short perfusions were carried out without heparin in the studies described in Sections 3C1-3.

C. Study of effects of subacute DBNP toxicity on FABP and STs

The rats utilized received daily intraperitoneal injections of 25mg/kg DBNP in dimethy sulfoxide (DBNP group) or the vehicle (controls). They were sacrificed in pairs (1 control + 1 DBNP rat) 33-58 days after the study began. The time period was shorter than planned, due to deaths in the DBNP group from day 30 on.

1. The DBNP-treated rats had enlarged livers Table 3 shows individual body weights, liver weights, and liver to body weight ratios of DBNP and control groups. Note that the body weights of the two groups were similar; the livers of DBNP-treated rats were larger than those of the controls; and liver to BW ratios of the groups differed significantly ($p<0.02$).

Table 3 Body weights(BWs), liver weights (LWs) and LW/BW of rats given DBNP or vehicle

Exptl Gp & No	BW(g)	LW(g)	Lw/BW
Controls(5)	283±16	9.71±0.57	0.0343±0.0011
DBNP (5)	257±17	11.,6±1.3	0.0446±0.0036
t test	-	-	P<0.02

2. FABP levels per g liver but not per 100g BW were decreased significantly by DBNP treatment Table 4 shows the FABP levels in mg FABP per g liver and per 100 g BW in DBNP and Control groups. Data were obtained after cytosol FABP was purified by consecutive DEAE-Sephadex A-50 and Sephadex g-75 chromatography (recall Figures 1 and 21). FABP per g liver decreased significantly by an average of 54% (P<0.2), while the data per 100 g BW showed only a nonsignificant 17% average decrease. The FABP data per 100 g BW (not shown) appeared to be biphasic, with the first three test pairs (days 33-45) differing by an average decrease of of 34% while the last two pairs (days 52-58) differed by a decrease of only 5%.

Table 4 Hepatic FABP levels in rats given DBNP or vehicle

Exptl Gp & No	Hepatic FABP in mg FABP per: g liver	100g BW
Controls(5)	0.743±0.095	4.09±0.57
DBNP (5)	0.341±0.085	3.41±0.74
Avg Effect	-54%	-17%
t test	P<0.02	n.s.

3. Bile salt sulfotransferase(BST) levels per 100 g BW, not per gliver, were increased significantly by DBNP and BSTI appeared to be involved Table 5 shows BST levels (nmol glycolithocholate sulfate, GLCS) made per g liver and per 100 g BW in DBNP or control rats. The data came from assay of BST activity with 4 levels of cytosol. BST activity per g liver did not increase in a statistically significant fashion. The increase of BST activity per 100 g BW averaged 71% (62-84%) and it was statistically significant.

Table 5 Hepatic BST levels in rats given DBNP or vehicle

Exptl Gp & No	Hepatic BST in nmol GLCS per: g liver	100g BW
Controls(5)	231±64	761±198
DBNP (5)	286±78	1300±310
Avg Effect	+24%	-71%
t test	n.s.	P<0.02

Figure 3 shows the results of chromatography of cytosol from a DBNP-treated rat and from a control rat on DEAE Sephadex A-50. As indicated in Methods the two equivalent liver samples were chromatographed side by side on nearly identical ion exchange columns. The data indicate that most of the BST activity in the DBNP-treated rat was due hepatic BST I, the main BST present in female rats(9). The control rat exhibited a typical "male" BST profile that contains lower levels of all three isofunctional BSTs. The BSTs are identified by the salt content of the highest effluent fraction of each enzyme peak. This was one of 3 quite similar experiments.

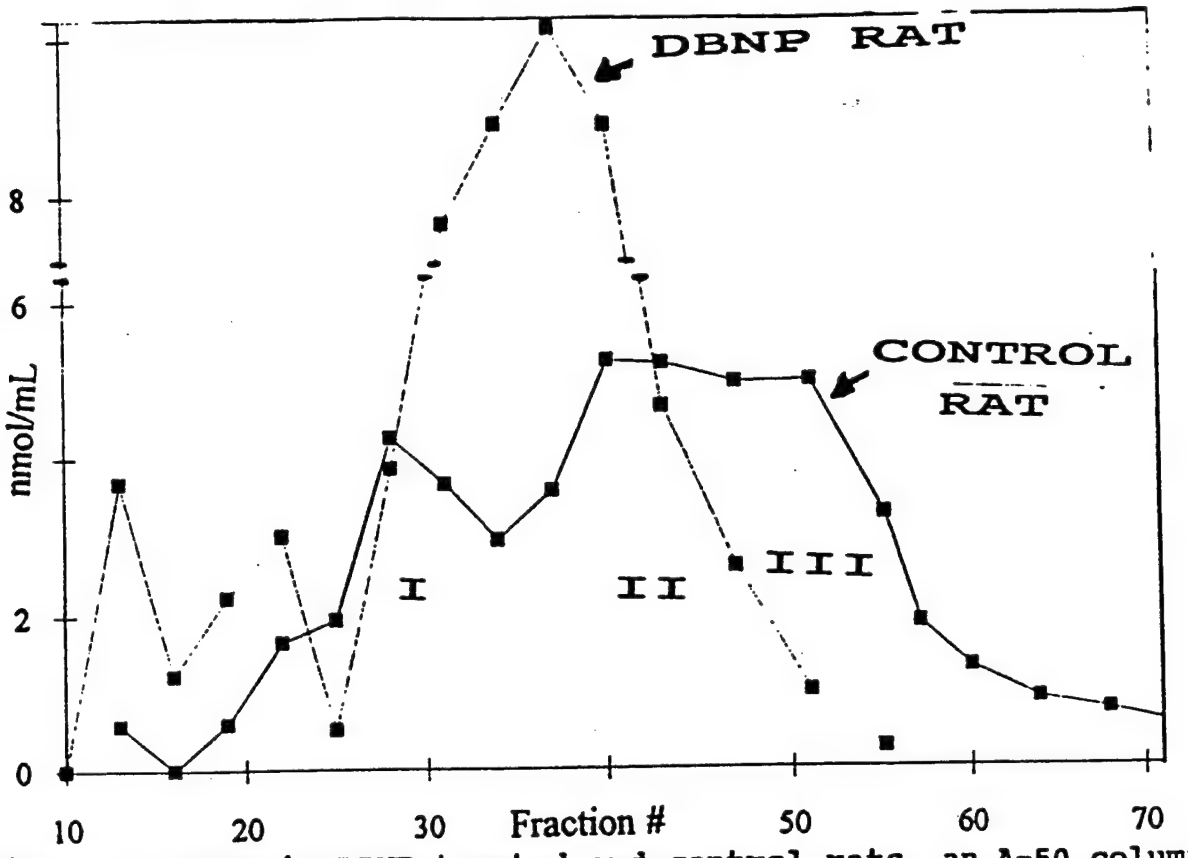

Figure 3. BSTs in DBNP-treated and control rats, an A-50 column

4. Dopamine sulfotransferase(DST) levels per g liver and per 100 g BW, were increased significantly by DBNP and DSTII appeared to be involved Table 6 shows DST levels (nmol dopamine sulfate, DS)per g liver and100 g BW in DBNP or control rats. Data came from assay of DST activity with 4 levels of cytosol. Activity per g liver and 100 g BW increased by an average of 31% and 28% (24-37%), respectively. The increases were statistically significant.

Table 6 Hepatic DST levels in rats given DBNP or vehicle

Exptl Gp & No	Hepatic DST, nmol DS present per: g liver	100g BW
Controls(5)	1530±260	4420±430
DBNP (4)	1170±250	5660±330
Avg Effect	+31%	+28%
t test	P<0.05	P<0.02

Chromatography of cytosol from a DBNP-treated rat and a control rat on DEAE Sephadex A-50 was also carried out(Figure 4). The figure depicts one of four similar experiments. The results indicate that most of the DST activity present in both rats is due to DSTII, the main enzyme we found in both males and females (21). Which enzyme is affected by DBNP administration, here, is presently unclear because of the small overall increase of total DST activity observed (recall Table 6).

Note that the identities of the DSTI and DSTII peaks in the two superimposed ion exchange chromatograms depicted are certain. They were ascertained from the effluent salt content of the peak tubes. There is no ambiguity in the data.

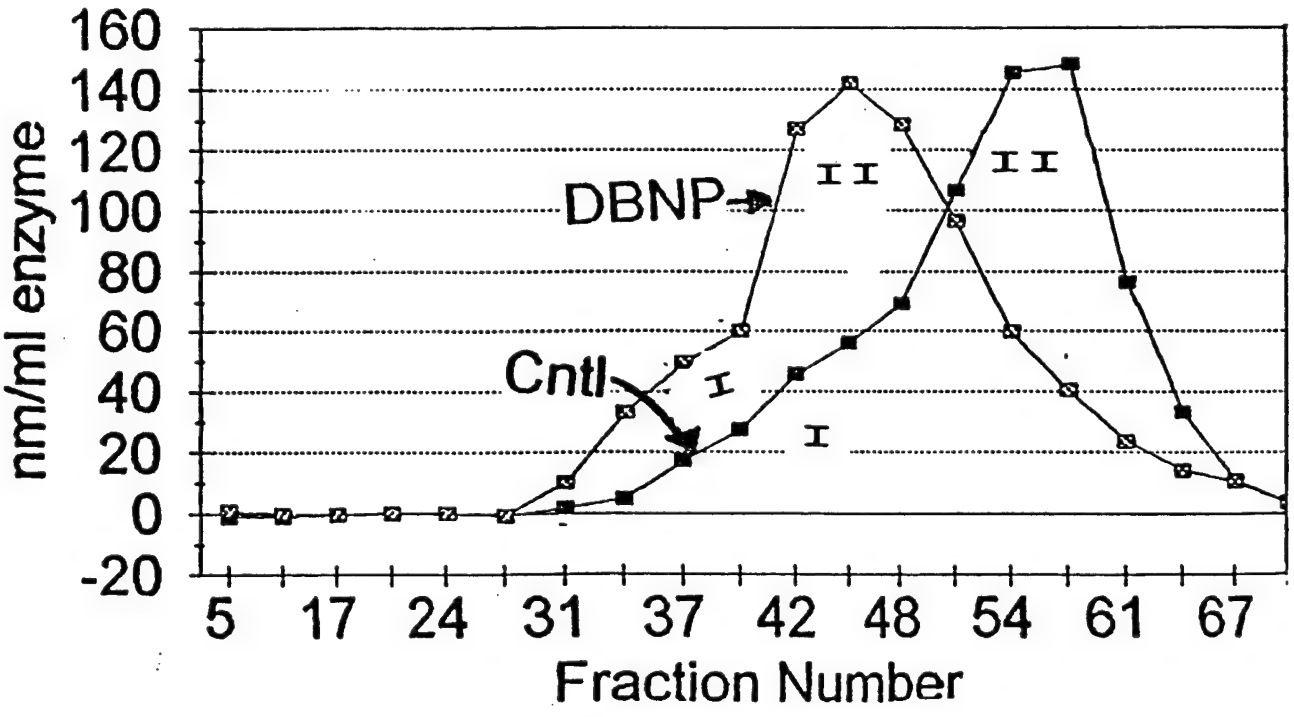

Figure 4. DSTs in DBNP-treated and control rats, as shown by DEAE-Sephadex A-50 chromatograms.

VI. DISCUSSION

The occurrence of DBNP on Naval submarines raises the specter of potential danger to personel that could accrue from this mammalian toxicant (1). The search for clues to such dangers is best begun with examination of parameters identified in animal study. Thus we sought to evaluate potential bases for toxicant action of DBNP in the rat, namely fatty liver and cardiovascular problems. We thus explored rat liver FABP, BST, EST, HCST and DST. FABP was a likely candidate for examination due to its importance in the biochemistry of lipids(7-13). The STs appeared relevant because altering hormone/emulsifier forms via sulfation would affect endocrine responses of the Phase I/II metabolic enzymes. In addition, DBNP seemed likely to interact with FABP and STs due to its structural resemblence to the substrates and inhibitors of these proteins.

Our first studies examined in vitro DBNP effects on FABP and the STs and the potential for DBNP sulfation by DST. We found that DBNP had no effect on FABP or DST and that DBNP was not sulfated either by cytosol or any DEAE Sephadex A-50 chromatograms we used to probe the individual STs. Hence:

1) in vitro interactions between the toxicant and our test parameters are deemed unlikely to obscure study of its biological effects
2) DBNP sulfation is not a probable occurrence in vivo and DBNP sulfate is an unlikely metabolite

Exploration of the effects of perfusion on the test parameters (Table 1) showed a small increase (averaging 25%) of the

isolated FABP and no effect on BST (Table 2) in livers perfused for several hours with 0.36 mM DBNP by the method used to probe other liver parameters at NMRI. Studies of DST and the steroid hormone sulfotransferases aborted due to methodological difficulties associated with the makeup of the perfusant (probably included heparin).

The perfusion data were not conclusive because of time, methodologic considerations and tissue available (only 2 animal sets) in the time frame agreed on. More samples should be looked at, if possible, due to need for resolution of these issues which in the case of DST and FABP seem likely with a few more samples. As to the other STs (HCST and EST), now that the methodologic complications are clear, additional stidies should be both possible and provide clarification.

The study of the effects of subacute DBNP toxicity on FABP and STs was most rewarding. In these rats, which developed enlarged -- presumably -- fatty livers (Table 3). FABP levels per g liver decreased significantly by an average of 54% within 33 days (Table 4). The data per 100 g BW showed what may be a biphasic response, presenting as an average 34% decrease between days 33 and 45 but only 5% between days 52 and 58. As this response could be crucial to development of fatty livers and to identification of long term hepatotoxicity additional animals should be examined. It is also possible that the actual effects were greater than seen because of the fact that the perfusion studies appeared to stabilize FABP. Hence future efforts must factor this observation into experimental plans. It is important

to continue this effort because it may yield valuable insight into understanding the potential diabetogenic complications of FABP raised by others(13) and cirrhotogenic potential that we have suggested (9).

The study of BST activity was also exciting. Though the BST levels did not rise significantly per g liver, a 71% increase per 100 g body weight was observed (Table 5) and this appeared to be due to alteration of relative levels of BSTI (Figure 3), the main BST present in female rats (9). This potential feminization of the BST in liver also supports endocrine alterations in the DBNP-treated rats that makes it important to explore the steroid sulfotransferases with which we were unsuccessful, now that we understand the problems involved. In addition, the BST data are in agreement with our expectations of BST-FABP interrelations (9) this may have cirrhotogenic implications we have suggested and may support the importance of the diminished FABP levels we report here.

Additional support for endocrine alterations arise from our observation of a small but significant elevation of the DST levels per g liver and per 100 g BW in DBNP rats (Table 6). Here, the catecholamine levels in vivo would be altered and lead us to suspect similar and perhaps more extensive alteration of EST and HCST that would modify tissue levels of glucocorticoids and estrogens.It is also important to identify the DST elevated, as DSTII increase would have different results from DSTI elevation (21).

VII. REFERENCES

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